

VACCINES FOR THE TREATMENT AND PREVENTION OF INFECTIOUS DISEASES IN INVERTEBRATES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. provisional application numbers 63/616,346, filed December 29, 2023; 63/643,343, filed May 6, 2024; 63/661,827, filed June 19, 2024; and 63/716,070, filed November 4, 2024, the content of each which is incorporated by reference into this application in its entirety.

TECHNICAL FIELD

[0002] This disclosure provides methods for one or more of: vaccinating, raising an immune response, treating, and/or preventing infections in invertebrates or a population of invertebrates such as insects, bees, crustaceans, and shrimp.

BACKGROUND

[0003] Driven by climate change and globalization, the spread of infectious diseases has become a growing global concern and is posing a growing public health concern as the spread is a threat to food security, access to nutrient rich foods, animal health, human healthcare, and plant health. Infectious diseases are caused by pathogenic microorganisms, such as bacteria, viruses, parasites, or fungi and can rapidly spread from one infected host, person, animal, or plant to another. Once an individual is infected, transfer can happen directly between individuals or via an intermediary vector host.

[0004] Infectious diseases are generally divided into three categories and are often dependent on infection level and predisposition of the host or patient. Those that can rapidly spread over long distances can cause high mortality and disability, and potentially global pandemics.

[0005] As the root cause of infectious diseases lays outside the infected host, individual person animal, or plant, human and animal healthcare and agriculture utilize three key strategies to lower the risk of infection: by immunization to increase the host's person or animal's resistance to an infection; by lowering the presence of the pathogens themselves or

the vector that carries them by prophylactic use of pesticides, antibiotics, or the release of vectors with lowered reproductive ability such as fruit flies or mosquitos.

[0006] Invertebrates such as honeybees and other insect pollinators, insects in general and crustaceans such as shrimp are critical part of our ecosystem, contribute to biodiversity, food security and climate concerns but are also threatened by diseases or are carriers of disease. Thus, new non-chemical and sustainable methods are needed to protect these animals rather than eradicating them. As such, a need exists in the art for effective vaccines for the prevention of disease in invertebrates and insects. This disclosure satisfies this need and provides related advantages as well.

SUMMARY OF THE DISCLOSURE

[0007] In this disclosure, Applicant provides novel compositions and/or methods to lower the infection burden in these important and underserved invertebrates by activating the invertebrates' innate immune system in the maternal and the next generation and thereby lowering their mortality and ability to pass on infections between them and across species.

[0008] *Treating and Preventing Bacterial and Viral Infections in Invertebrates*

[0009] Applicant provides a method for one or more of: vaccinating, raising an immune response, treating, and/or preventing disease or infections in an invertebrate or a population of invertebrates against a microbial (e.g., bacterial and/or viral) infection or a microbial or a viral disease, the method comprising, or consisting essentially of, or yet further consisting of, administering an effective amount of dead and/or inactivated non-disease causing bacterial species or fragments thereof and optionally combined with a carrier, to the invertebrate, thereby vaccinating, treating, preventing or immunizing the invertebrate against the microbial disease or infection. In one aspect the maternal invertebrate is administered the composition and the immune response, treatment, and/or prevention of the disease or infection is based to the offspring. In one aspect, the invertebrate or population thereof is identified in **Table 1**, or alternatively, **Tables 1-4** and **Experiment No. 4**, e.g., an insect (a bee such as a honey bee or a bumble bee), a crustacean, or a shrimp and exemplary dead and/or inactivated non-disease causing bacterial species or fragments thereof are listed in the tables. Non-limiting examples

of invertebrates include bees, honey bees, bumble bees, crustaceans, shrimp, fleas, ticks, flies, mites, mosquitos, and populations thereof.

[0010] In one aspect, the dead and/or inactivated non-disease causing bacterial species comprises one or more gram-positive bacteria and/or one or more gram-negative bacteria. In another aspect, the dead and/or inactivated non-disease causing bacterial species comprises, or consists essentially of, or yet further consist of one or more gram-positive bacteria.

[0011] To the best of Applicant's knowledge, this is the first vaccine that provides invertebrates, crustacean such as shrimp, and insects such as honey bees protection, their offspring and populations thereof against any viral disease or non-disease causing bacterial or viral infection and the first example of trans generational priming (TGIP) using a bacterial pathogen that provides protection against a virus and non-disease causing bacteria in invertebrates such as insects, bees, crustaceans, and shrimp. In another aspect, the method is applied to the specific invertebrates and diseases identified in **Table 1**, or alternatively **Tables 1-4**, or as identified in **Experiment No. 4**.

[0012] In one aspect, the disclosure provides methods one or more of: vaccinating, raising an immune response, treating, and/or preventing disease caused by infection by viral and/or bacterial pathogens in invertebrates or invertebrate populations such as insects, bees, crustaceans, and shrimp. The methods comprise, or consist essentially of, or consist of administering and/or immunizing the invertebrate by administering to, feeding to, or applying a stimulus comprising a dead and/or inactivated non-disease causing gram positive bacterial species to the invertebrate or an invertebrate colony to immunize the invertebrate or its offspring.

[0013] In one aspect of the disclosed methods, the dead and/or inactivated non-disease causing gram-positive bacteria or cell wall fragments thereof that are administered comprise, or consist essentially of, or consist of, a dead and/or inactivated bacterial species as disclosed in **Table 1**, or alternatively **Tables 1-4** or of the genus *Paenibacillus*, for example selected from *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericanus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*,

P. chinjuensis, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdolanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvificiens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynnii*, and/or *P. xylanilyticus* or a combination of two or more thereof or *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvans*, or a combination of two or more thereof. In yet another aspect, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises, or consists essentially thereof, or consists of a dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof.

[0014] In a further aspect, the composition that is administered in the method further comprises, or consists essentially of, or yet further consists of an effective amount of a gram negative dead and/or inactivated non-disease-causing bacteria or a cell wall fragment thereof of a *Vibrio* sp., e.g., selected from *V. adaptatus*, *V. aerogenes*, *V. aestivus*, *V. aestuariamus*, *V. agarivorans*, *V. albensis*, *V. alfacensis*, *V. alginolyticus*, *V. anguillarum*, *V. areninigras*, *V. artabrorum*, *V. atlanticus*, *V. atypicus*, *V. azureus*, *V. brasiliensis*, *V. bubulus*, *V. calviensis*, *V. campbellii*, *V. casei*, *V. chagasii*, *V. cholerae*, *V. cincinnatiensis*, *V. coralliilyticus*, *V. crassostreae*, *V. cyclitrophicus*, *V. diabolicus*, *V. diazotrophicus*, *V. ezurae*, *V. fluvialis*, *V. fortis*, *V. furnissii*, *V. gallicus*, *V. gazogenes*, *V. gigantis*, *V. haliotocoli*, *V.*

harveyi, *V. hepatarius*, *V. hippocampi*, *V. hispanicus*, *V. ichthyoenteri*, *V. indicus*, *V. kanaloae*, *V. lentus*, *V. litoralis*, *V. logei*, *V. mediterranei*, *V. metschnikovii*, *V. mimicus*, *V. mytili*, *V. natrigens*, *V. navarrensis*, *V. neonatus*, *V. neptunius*, *V. nereis*, *V. nigripulchritudo*, *V. ordalii*, *V. orientalis*, *V. pacinii*, *V. parahaemolyticus*, *V. pectenica*, *V. pelagius*, *V. penaeicida*, *V. pomeroyi*, *V. ponticus*, *V. proteolyticus*, *V. rotiferianus*, *V. ruber*, *V. rumoiensis*, *V. salmonicida*, *V. scophthalmi*, *V. splendidus*, *V. superstes*, *V. tapetis*, *V. tasmaniensis*, *V. tubiashii*, *V. vulnificus*, *V. wodanis*, and/or *V. xuii* or a combination of two or more thereof, or alternatively selected from the group consisting of *Vibrio alginolyticus*, *V. anguillarum*, *V. campbelli*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*, *V. penaeicida*, *V. vulnificus*, *V. nereis*, *V. tubiashi*, *V. fluvialis*, *V. splendidus*, *V. nigripulchritudo*, *Hepatobacter penaeiis*, *Vibrio alginolyticus*, *V. anguillarum*, *V. campbelli*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*. In a further aspect, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises, or consists essentially thereof, or consists of a dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof and are administered in combination with a dead and/or inactivated non-disease causing bacteria or a cell wall fragment thereof of a *Vibrio* sp.

[0015] *Treating and Preventing Viral Infections in Bees and Bee Populations*

[0016] In contrast to what had been known in the art and by way of example only, Applicant describes in one embodiment a method that comprises, or consists essentially of, or yet further consists of administration, feeding and/or immunization of invertebrates by applying a stimulus comprising a dead and/or inactivated non-disease causing gram positive bacteria or a bacterial species, (e.g., *Paenibacillus larvae* (PL)) or cell wall fragments thereof to the invertebrate for the protection against infection by viral pathogens in the invertebrate or their offspring. Non-limiting examples of such invertebrates include shrimp, insects, crustaceans, and honey bees. For example, Applicant has shown that administration of a vaccine comprising the dead non-disease causing bacterial pathogen *Paenibacillus larvae* (PL) to honey bees reduces deformed wing virus B (DWV-B) quantities in a honey bee colony, i.e., the queen bee and the offspring of the vaccinated bee. Thus, this vaccine provides beekeepers with a new method of reducing the impact of viral infections caused by *Varroa destructor* mite infestation on their honey bee colonies, and also improves colony health by decreasing

the quantities of DWV-B in the colony. Accordingly, the present disclosure provides these preventive and therapeutic methods.

[0017] Thus, in one aspect, this disclosure provides a method for one or more of: vaccinating, treating, preventing or immunizing a bee or a population of bees against a viral infection or viral disease the method comprising, or consisting essentially of, or yet further consisting of, administering an effective amount of dead and/or inactivated gram-positive bacteria, e.g., *Paenibacillus larvae* (PL) or fragments such as cell wall fragments thereof and optionally combined with a carrier to the bee or the population of bees, thereby vaccinating, treating or immunizing the bee or bee population against the viral disease or infection. In one aspect, the queen bee is vaccinated and as a result, the queen and the offspring are vaccinated, treated, or immunized against the viral infection or viral disease.

[0018] In one aspect, the bee is a honey bee or population of bees is a honey bee population or colony and the virus causing the viral disease or infection is selected from Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus. In another aspect, the bee is a bumble bee or a bumble bee population or colony and wherein the viral disease or infection is caused by Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus. In a further aspect, the bee is a honey bee and the population is a honey bee population or colony, and the virus causing the disease or infection is Deformed Wing Virus-B (DWV-B).

[0019] In one embodiment of the above disclosure, the carrier comprises a bee food or a bee feed.

[0020] In one aspect of the above methods, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprise, or consist essentially of, or consists of a dead and/or inactivated bacterial species of **Table 1** or, for example, of the genus *Paenibacillus*. In another aspect, the dead and/or inactivated bacterial *Paenibacillus* species

is selected from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericamus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvificiens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynmii*, and/or *P. xylanilyticus* or a combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvens*, or a combination of two or more thereof. In yet another aspect, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof.

[0021] In a further aspect, the composition that is administered in the method further comprises, or consists essentially of, or yet further consists of an effective amount of a gram negative dead and/or inactivated non-disease-causing bacteria or a cell wall fragment thereof of a *Vibrio* sp., e.g., selected from *V. adaptatus*, *V. aerogenes*, *V. aestivus*, *V. aestuariamus*, *V. agarivorans*, *V. albensis*, *V. alfacensis*, *V. alginolyticus*, *V. anguillarum*, *V. areninigræ*,

V. artabrorum, *V. atlanticus*, *V. atypicus*, *V. azureus*, *V. brasiliensis*, *V. bubulus*, *V. calviensis*, *V. campbellii*, *V. casei*, *V. chagasii*, *V. cholerae*, *V. cincinnatiensis*, *V. coralliilyticus*, *V. crassostreae*, *V. cyclitrophicus*, *V. diabolicus*, *V. diazotrophicus*, *V. ezurae*, *V. fluvialis*, *V. fortis*, *V. furnissii*, *V. gallicus*, *V. gazogenes*, *V. gigantis*, *V. haliotocoli*, *V. harveyi*, *V. hepatarius*, *V. hippocampi*, *V. hispanicus*, *V. ichthyenteri*, *V. indicus*, *V. kanaloae*, *V. lentus*, *V. litoralis*, *V. logei*, *V. mediterranei*, *V. metschnikovii*, *V. mimicus*, *V. mytili*, *V. natriegens*, *V. navarrensis*, *V. neonatus*, *V. neptunius*, *V. nereis*, *V. nigripulchritudo*, *V. ordalii*, *V. orientalis*, *V. pacinii*, *V. parahaemolyticus*, *V. pectenocida*, *V. pelagius*, *V. penaeocida*, *V. pomeroyi*, *V. ponticus*, *V. proteolyticus*, *V. rotiferianus*, *V. ruber*, *V. rumoiensis*, *V. salmonocida*, *V. scophthalmi*, *V. splendidus*, *V. superstes*, *V. tapetis*, *V. tasmaniensis*, *V. tubiashii*, *V. vulnificus*, *V. wodanis*, and/or *V. xuii* or a combination of two or more thereof, or alternatively selected from the group consisting of *Vibrio alginolyticus*, *V. anguillarum*, *V. campbelli*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*, *V. penaeocida*, *V. vulnificus*, *V. nereis*, *V. tubiashi*, *V. fluvialis*, *V. splendidus*, *V. nigripulchritudo*, *Hepatobacter penaeiis*, *Vibrio alginolyticus*, *V. anguillarum*, *V. campbelli*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus* or a combination of two or more thereof. In a further aspect, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises, or consists essentially thereof, or consists of a dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof and are administered in combination with a dead and/or inactivated non-disease causing bacteria or a cell wall fragment thereof of a *Vibrio* sp.

[0022] *Treating and Preventing Bacterial and Viral Infections in Crustaceans*

[0023] Further provided is a method for one or more of: vaccinating, raising an immune response, treating, and/or preventing disease caused by infection by viral and/or bacterial pathogens in crustaceans and crustacean populations. The methods comprise, or consist essentially of, or consist of administering and/or immunizing the crustacean or population by administering to, feeding to, or applying a stimulus to the crustacean or population a dead and/or inactivated non-disease causing gram negative bacterial species or cell wall fragments thereof to the crustacean or population. In one aspect, the crustacean, or population thereof is a shrimp. In another aspect, the crustacean is a shrimp but with the proviso that the shrimp or population thereof to be vaccinated does not include *P. polymyxa* for the vaccination of

shrimp and their offspring against *V. parahaemolyticus* infections or disease, when *P. polymyxa* is the sole active agent in the vaccine.

[0024] In a further aspect, the composition that is administered in the method comprises, or consists essentially of, or yet further consists of an effective amount of a gram negative dead and/or inactivated non-disease-causing bacteria or a cell wall fragment thereof of a *Vibrio* sp., e.g., selected from *V. adaptatus*, *V. aerogenes*, *V. aestivus*, *V. aestuarianus*, *V. agarivorans*, *V. albensis*, *V. alfacensis*, *V. alginolyticus*, *V. anguillarum*, *V. areninigræ*, *V. artabrorum*, *V. atlanticus*, *V. atypicus*, *V. azureus*, *V. brasiliensis*, *V. bubulus*, *V. calviensis*, *V. campbellii*, *V. casei*, *V. chagasii*, *V. cholerae*, *V. cincinnatiensis*, *V. coralliilyticus*, *V. crassostreae*, *V. cyclitrophicus*, *V. diabolicus*, *V. diazotrophicus*, *V. ezurae*, *V. fluvialis*, *V. fortis*, *V. furnissii*, *V. gallicus*, *V. gazogenes*, *V. gigantis*, *V. haliotocoli*, *V. harveyi*, *V. hepatarius*, *V. hippocampi*, *V. hispanicus*, *V. ichthyenteri*, *V. indicus*, *V. kanaloae*, *V. lentus*, *V. litoralis*, *V. logei*, *V. mediterranei*, *V. metschnikovii*, *V. mimicus*, *V. mytili*, *V. natriegens*, *V. navarrensis*, *V. neonatus*, *V. neptunius*, *V. nereis*, *V. nigripulchritudo*, *V. ordalii*, *V. orientalis*, *V. pacinii*, *V. parahaemolyticus*, *V. pectenica*, *V. pelagius*, *V. penaeicida*, *V. pomeroyi*, *V. ponticus*, *V. proteolyticus*, *V. rotiferianus*, *V. ruber*, *V. rumoiensis*, *V. salmonicida*, *V. scopthalmi*, *V. splendidus*, *V. superstes*, *V. tapetis*, *V. tasmaniensis*, *V. tubiashii*, *V. vulnificus*, *V. wodanis*, and/or *V. xuii* or a combination of two or more thereof, or alternatively selected from the group consisting of *Vibrio alginolyticus*, *V. anguillarum*, *V. campbellii*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*, *V. penaeicida*, *V. vulnificus*, *V. nereis*, *V. tubiashi*, *V. fluvialis*, *V. splendidus*, *V. nigripulchritudo*, *Hepatobacter penaeiis*, *Vibrio alginolyticus*, *V. anguillarum*, *V. campbellii*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*, or a combination of two or more thereof.

[0025] In a further aspect, the composition that is administered in the method comprises, or consists essentially of, or yet further consists of an effective amount of a gram negative dead and/or inactivated non-disease-causing bacteria or a cell wall fragment thereof of a *Vibrio* sp., e.g., selected from *V. adaptatus*, *V. aerogenes*, *V. aestivus*, *V. aestuarianus*, *V. agarivorans*, *V. albensis*, *V. alfacensis*, *V. alginolyticus*, *V. anguillarum*, *V. areninigræ*, *V. artabrorum*, *V. atlanticus*, *V. atypicus*, *V. azureus*, *V. brasiliensis*, *V. bubulus*, *V. calviensis*, *V. campbellii*, *V. casei*, *V. chagasii*, *V. cholerae*, *V. cincinnatiensis*, *V. coralliilyticus*, *V. crassostreae*, *V.*

cyclitrophicus, *V. diabolicus*, *V. diazotrophicus*, *V. ezurae*, *V. fluvialis*, *V. fortis*, *V. furnissii*, *V. gallicus*, *V. gazogenes*, *V. gigantis*, *V. halioticoli*, *V. harveyi*, *V. hepatarius*, *V. hippocampi*, *V. hispanicus*, *V. ichthyoenteri*, *V. indicus*, *V. kanaloae*, *V. lentus*, *V. litoralis*, *V. logei*, *V. mediterranei*, *V. metschnikovii*, *V. mimicus*, *V. mytili*, *V. natriegens*, *V. navarrensis*, *V. neonatus*, *V. neptunius*, *V. nereis*, *V. nigripulchritudo*, *V. ordalii*, *V. orientalis*, *V. pacinii*, *V. parahaemolyticus*, *V. pectenica*, *V. pelagius*, *V. penaeicida*, *V. pomeroyi*, *V. ponticus*, *V. proteolyticus*, *V. rotiferianus*, *V. ruber*, *V. rumoiensis*, *V. salmonicida*, *V. scopthalmi*, *V. splendidus*, *V. superstes*, *V. tapetis*, *V. tasmaniensis*, *V. tubiashii*, *V. vulnificus*, *V. wodanis*, and/or *V. xuii* or a combination of two or more thereof, or alternatively selected from the group consisting of *Vibrio alginolyticus*, *V. anguillarum*, *V. campbelli*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*, *V. penaeicida*, *V. vulnificus*, *V. nereis*, *V. tubiashi*, *V. fluvialis*, *V. splendidus*, *V. nigripulchritudo*, *Hepatobacter penaeiis*, *Vibrio alginolyticus*, *V. anguillarum*, *V. campbelli*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*, or a combination of two or more thereof. In a further aspect, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises, or consists essentially thereof, or consists of a dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof and are administered in combination with a dead and/or inactivated non-disease causing bacteria or a cell wall fragment thereof of a *Vibrio* sp.

[0026] In a further aspect, the crustacean or population is a shrimp or shrimp population and the infection or disease being treated is White Spot Syndrome Virus (WSSV) in shrimp and an effective amount of PL alone or in combination with *Vibrio parahaemolyticus* (VP). The non-disease causing bacteria can be administered as known in the art, e.g., in a feed or by injection.

[0027] *Treating and Preventing Bacterial and Viral and Bacterial Infections in Culicinae and Anophelinae*

[0028] Also provided herein are compositions and methods for a novel approach for immunizing and treating Culicidae (mosquito) species and/or its host against non-disease causing bacteria or viral infection or disease, wherein the Culicidae include, for example the subfamilies Culicinae and Anophelinae with an effective amount of one or more dead and/or heat-inactivated gram positive bacteria or cell wall fragments thereof. See, for example

Tables 2 and 3 for a list of diseases and hosts. Non-limiting examples of Culicidae species include *Aedes albopictus*, *Aedes aegypti*, and *Aedes polynesiensis*. Thus, provided herein are compositions and methods for one or more of: vaccinating, treating, preventing or immunizing a Culicidae and/or their offspring and/or the Culicidae host against a viral infection, bacterial infection, bacterial disease or viral disease, and separately transmission of the disease, the method comprising, or consisting essentially of, or yet further consisting of, administering an effective amount of dead and/or inactivated gram-positive bacteria, e.g., *Paenibacillus larvae* (PL) or fragments such as cell wall fragments thereof, and optionally combined with a carrier to the Culicidae or the Culicidae host, thereby vaccinating, treating or immunizing the Culicidae, the offspring, and/or the host against the viral or bacterial disease or infection. The dead and/or inactivated gram positive bacteria are described herein, the complete list of which is incorporated by reference herein. Non-limiting examples of the group includes one or more *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericanus*, *P. antarcticus*, *P. apiaris*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvificiens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynmii*, and/or *P. xylanilyticus* or a combination of two or more thereof.

[0029] In another aspect, the dead and/or inactivated *Paenibacillus* bacterial species is selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*,

Paenibacillus humicus, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvens*, or a combination of two or more thereof.

[0030] In one aspect, the dead and/or inactivated bacteria is a *Paenibacillus sp.*, e.g., PL. The dead and/or inactivated *Paenibacillus sp.* bacteria or cell wall fragments thereof can be feed to the Culicidae.

[0031] Compositions

[0032] Compositions comprising, or consisting essentially of, or yet further consisting of a dead and/or inactivated gram positive or gram negative non-disease causing bacteria or a cell wall fragment thereof of gram-positive bacteria or cell wall fragments thereof. In one aspect the non-disease causing bacteria is a gram positive bacterium of the genus *Paenibacillus*. In one aspect, the dead and/or inactivated *Paenibacillus* bacterial species is selected from the group consisting of *P. agarixedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericanus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlandolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvifaciens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynmii*, and/or *P. xylanilyticus* or a combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus*

dentritiformis, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvens*, or a combination of two or more thereof. In yet another aspect, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof.

[0033] Alternatively the composition comprises, or consists essentially of, or yet further consists of a non-disease causing bacteria is a gram negative bacterium of a *V. adaptatus*, *V. aerogenes*, *V. aestivus*, *V. aestuariamus*, *V. agarivorans*, *V. albensis*, *V. alfacensis*, *V. alginolyticus*, *V. anguillarum*, *V. areninigrae*, *V. artabrorum*, *V. atlanticus*, *V. atypicus*, *V. azureus*, *V. brasiliensis*, *V. bubulus*, *V. calviensis*, *V. campbellii*, *V. casei*, *V. chagasii*, *V. cholerae*, *V. cincinnatiensis*, *V. coralliilyticus*, *V. crassostreae*, *V. cyclitrophicus*, *V. diabolicus*, *V. diazotrophicus*, *V. ezurae*, *V. fluvialis*, *V. fortis*, *V. furnissii*, *V. gallicus*, *V. gazogenes*, *V. gigantis*, *V. haliotocoli*, *V. harveyi*, *V. hepatarius*, *V. hippocampi*, *V. hispanicus*, *V. ichthyenteri*, *V. indicus*, *V. kanaloae*, *V. lentus*, *V. litoralis*, *V. logei*, *V. mediterranei*, *V. metschnikovii*, *V. mimicus*, *V. mytili*, *V. natriegens*, *V. navarrensis*, *V. neonatus*, *V. neptunius*, *V. nereis*, *V. nigripulchritudo*, *V. ordalii*, *V. orientalis*, *V. pacinii*, *V. parahaemolyticus*, *V. pectenica*, *V. pelagius*, *V. penaeicida*, *V. pomeroyi*, *V. ponticus*, *V. proteolyticus*, *V. rotiferianus*, *V. ruber*, *V. rumoiensis*, *V. salmonicida*, *V. scopthalmi*, *V. splendidus*, *V. superstes*, *V. tapetis*, *V. tasmaniensis*, *V. tubiashii*, *V. vulnificus*, *V. wodanis*, and/or *V. xiii* or a combination of two or more thereof, or alternatively selected from the group consisting of *Vibrio* sp., e.g. selected from *Hepatobacter penaeiis*, *Vibrio alginolyticus*, *V. anguillarum*, *V. campbelli*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*, *V. penaeicida*, *V. vulnificus*, *V. nereis*, *V. tubiashi*, *V. fluvialis*, *V. splendidus*, *V. nigripulchritudo* alone or in combination with a non-disease causing dead and/or inactivated gram positive bacteria is

provided. In one embodiment, the dead or inactivated gram positive bacteria is a PL or a cell wall fragment thereof in combination with the *Vibrio sp.* These compositions can further comprise a carrier, such as an invertebrate food, e.g., shrimp feed and used for the treatment of shrimp.

[0034] Also provided herein is a composition comprising, or consisting essentially of, or yet further consisting of dead and/or inactivated whole cells or cell wall fragments of a gram-negative bacteria selected from *V. adaptatus*, *V. aerogenes*, *V. aestivus*, *V. aestuariamus*, *V. agarivorans*, *V. albensis*, *V. alfacensis*, *V. alginolyticus*, *V. anguillarum*, *V. areninigræ*, *V. artabrorum*, *V. atlanticus*, *V. atypicus*, *V. azureus*, *V. brasiliensis*, *V. bubulus*, *V. calviensis*, *V. campbellii*, *V. casei*, *V. chagasii*, *V. cholerae*, *V. cincinnatiensis*, *V. coralliilyticus*, *V. crassostreae*, *V. cyclitrophicus*, *V. diabolicus*, *V. diazotrophicus*, *V. ezurae*, *V. fluvialis*, *V. fortis*, *V. furnissii*, *V. gallicus*, *V. gazogenes*, *V. gigantis*, *V. halioticoli*, *V. harveyi*, *V. hepatarius*, *V. hippocampi*, *V. hispanicus*, *V. ichthyenterii*, *V. indicus*, *V. kanaloae*, *V. lentus*, *V. litoralis*, *V. logei*, *V. mediterranei*, *V. metschnikovii*, *V. mimicus*, *V. mytili*, *V. natriegens*, *V. navarrensis*, *V. neonatus*, *V. neptunius*, *V. nereis*, *V. nigripulchritudo*, *V. ordalii*, *V. orientalis*, *V. pacinii*, *V. parahaemolyticus*, *V. pectenica*, *V. pelagius*, *V. penaeicida*, *V. pomeroyi*, *V. ponticus*, *V. proteolyticus*, *V. rotiferianus*, *V. ruber*, *V. rumoiensis*, *V. salmonicida*, *V. scophthalmi*, *V. splendidus*, *V. superstes*, *V. tapetis*, *V. tasmaniensis*, *V. tubiashii*, *V. vulnificus*, *V. wodanis*, and/or *V. xuii* or a combination of two or more thereof, or alternatively selected from the group consisting of *Vibrio alginolyticus*, *V. anguillarum*, *V. campbellii*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*, *V. penaeicida*, *V. vulnificus*, *V. nereis*, *V. tubiashi*, *V. fluvialis*, *V. splendidus*, *V. nigripulchritudo*, or a combination of two or more thereof and a gram positive bacterial vaccine of **Table 1** and a carrier. In one aspect, the carrier comprises a feed for an invertebrate such as a shrimp. In another aspect, the composition is formulated for oral administration, administration by injection, and/or administration by immersion for the invertebrate, or combinations thereof. In another aspect, the invertebrate is identified in **Tables 1-4** and **Experiment No. 4**.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] **FIG. 1:** graphically illustrates an exemplary method to obtain bacterial fragments to be used as antigens. This figure is reproduced from Lodish, H. (ed) Molecular Cell Biology,

Sixth Edition, 2008 W.H. Freeman and Company.

[0036] FIG. 2: Box and whisker plot showing DWV-B quantities as measured by PCR analysis. Samples of nurse honey bee colonies were taken from colonies one week pre-vaccination and 4 months post-vaccination. In each of our 8 yards, one sample was submitted from colonies with vaccinated queens and one sample from colonies with unvaccinated queens (N=8 control, N=8 vaccinated). In each sample, 50 nurse bees were pooled evenly from 10 hives of the same treatment group. Prior to vaccination, there were no differences in DWV-B levels between treatment groups (Wilcoxon rank sum test, $W=31$, $P=0.96$). Post vaccination, vaccinated colonies had significantly reduced loads of DWV-B compared to control colonies (Wilcoxon rank sum test, $W=54.5$, $P=0.021$). Significance ($P<0.05$) on a Wilcoxon rank sum test is denoted by (*) between bar graphs.

[0037] FIG. 3: Box and whisker plot showing Varroa destructor mite count in vaccinated and unvaccinated honey bee colonies one week before (N=35 unvaccinated, N=38 vaccinated) and six months after (N=44 vaccinated and N=44 unvaccinated) vaccination. Counts were taken by performing a standard alcohol mite wash. A Welch two sample t-test showed there was no difference in mite quantities between groups either before ($T=0.38$, $df=61$ $P=0.71$) or after ($T=0.38$, $df=61$ $P=0.71$) vaccination.

[0038] FIGS. 4A – 4B: shows a comparison of the survival rate of vaccinated (with *P. larvae* (PL) and/or *V. parahaemolyticus* (VP)) and unvaccinated shrimp challenged with either AHPND (**FIG. 4A**) or WSSV (**FIG. 4B**) separately. (**FIG. 4A**) Survival at 6 days post challenge of next generation shrimp with EMS at a size of 10g after vaccination/treatment of female brood stock with PL and PL/PV combination product. (**FIG. 4B**) Survival at 12 days post challenge of next generation shrimp with WSSV at a size of 2g after vaccination/treatment of female brood stock with PL and PL/PV combination product.

[0039] FIGS. 5A- 5B: show injection of shrimp. Vaccine is delivered to the female broodstock by injection (**FIG. 5A**). Briefly, shrimp are removed from their tanks for injection. Treatments are administered via intra-muscular (IM) injection per ovulation cycle into the ventral axis between pereopods and pleopods of 100 μ L per shrimp. (**FIG. 5B**) A 0.3

mL insulin syringe (30G) with the needle length 8mm (5/6 inch) can be used. Once injected, shrimp will be placed back into the tanks.

[0040] **FIGS. 6A-6C:** Artificial feeding systems for mosquitoes. (**FIG. 6A**) *Aedes aegypti* mosquitoes engorged on an artificial blood meal replacement diet. Food colors were added to the different SkitoSnacks; (**FIG. 6B**) Glass membrane feeder for mosquitoes. Warm water is used in this device to keep the meal at body temperature. Mosquitoes suck the meal through a Parafilm® membrane (Sigma Aldrich, St. Louis, MO, USA); (**FIG. 6C**) Hemotek feeding system (Hemotek Ltd., Great Harwood, UK). Reproduced from <https://www.mdpi.com/1660-4601/13/12/1267>.

DETAILED DESCRIPTION

[0041] Definitions

[0042] As used herein and in the appended claims, singular articles such as "a" and "an" and "the" and similar referents in the context of describing the elements are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

[0043] As used herein, "about" is understood by persons of ordinary skill in the art and may vary to some extent depending upon the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which the term "about" is used, "about" will mean up to plus or minus 10% of the particular term.

[0044] As will be understood by one skilled in the art, for any and all purposes, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Furthermore, as will be understood by one skilled in the art, a range includes each individual member.

[0045] The term "exemplary" as used herein refers to "serving as an example, instance, or illustration," and not "preferred" or "advantageous over other embodiments."

[0046] The terms "non-disease causing" species or "non-disease species" as used herein refer to species of bacteria which may or may not be pathogenic but which do not cause the infectious disease, e.g., viral disease being targeted, treated and/or prevented.

[0047] An “invertebrate” intends an animal that neither develops nor retains a vertebral column, which evolved from the notochord. It is a paraphyletic grouping including all animals excluding the chordate subphylum Vertebrata, i.e., vertebrates.

[0048] As used herein, the term “host” intends the animal that is infected with the virus or bacteria, whether or not the animal becomes ill from harboring the virus or bacteria.

[0049] An “insect” is hexapod invertebrate of the class Insecta. They are the largest group within the arthropod phylum. Insects have a chitinous exoskeleton, a three-part body, three pairs of jointed legs, compound eyes, and a pair of antennae. Non-limiting examples of such include arachnids, bees, mites, ticks, mosquitoes, flies, and those identified in **Table 1**, or alternatively **Tables 1-4** and **Experiment No. 4**.

[0050] A “mite” is a minute arachnid which has four pairs of legs when adult, related to the ticks. Many kinds live in the soil and a number are parasitic on plants or animals.

[0051] A “fly” is an insect of the order “Diptera” that use a single pair of wings to fly. Diptera is a large order containing more than 150,000 species, including horse flies, fruit flies, house flies, tsetse flies, screwworms, crane flies, hoverflies, mosquitoes, and others.

[0052] A “mosquito” or “Culicidae” intends a family of small flies that consists of 3,600 species. They have a slender segmented body, one pair of wings, three pairs of long hair-like legs, and specialized, highly elongated piercing-suckling mouthparts. The mosquito's saliva is transferred to the host during the bite and can cause an itchy rash. In addition, blood-feeding species can ingest pathogens while biting, and transmit them to other hosts. Those species include vectors of parasitic diseases such as malaria and filariasis, and arboviral diseases such as yellow fever and dengue fever. By transmitting diseases, mosquitoes cause the deaths of over 725,000 people each year.

[0053] A “crustacean” intends are invertebrate animals that constitute one group of arthropods that are a part of the subphylum Crustacea, a large, diverse group of mainly aquatic arthropods including decapods, (shrimps, prawns, crabs, lobsters and crayfish), seed shrimp, branchiopods, fish lice, krill, remipedes, isopods, barnacles, copepods, opossum shrimps, amphipods and mantis shrimp.

[0054] A “shrimp” intends a crustacean with an elongated body and a primarily swimming mode of locomotion. Shrimp typically belonging to the Caridea or Dendrobranchiata of the decapod order, although some crustaceans outside of this order are also referred to as “shrimp.” Non-limiting examples of shrimp include Rock Shrimp, Pink Shrimp, Tiger Shrimp, Chinese White Shrimp, Brown Shrimp, White Shrimp, Atlantic Northern Shrimp, and Spot Shrimp.

[0055] The term “honey bee” as used herein refers to is any bee which is a member of the genus *Apis*, primarily distinguished by the production and storage of honey and the construction of perennial, colonial nests from wax. For example, two species of honey bees, namely *A. mellifera* or *A. cerana indica*, are often maintained by beekeepers. Honey bees include but are not limited to *Apis andreniformis* and *Apis florea* in subgenus *Micrapis*, *Apis dorsata* in subgenus *Megapis*, and *Apis cerana*, *Apis koschevnikovi*, *Apis mellifera* and *Apis nigrocincta* in subgenus *Apis*.

[0056] The term “bee colony” or “honey bee colony” or “bumble bee colony” as used herein, refers to a social unit of bees, e.g., honey or bumble bees comprising a colony also referred to herein as a population. The social unit can be of any system organization utilized by bees, which has the purpose of facilitating survival of the group or colony. Typically, a “bee colony” consists of several thousand bees that cooperate in nest building, food collection, and brood rearing. Each member of a “bee colony” has a definite task to perform, and it takes the combined efforts of the entire colony to survive and reproduce. A bee colony typically comprises a single queen, thousands of workers, and hundreds of drones during late spring and summer. Typically a “bee colony” consists of highly related bees, with colony size depending on the species (ex. several thousand individuals for honey bees, and several hundred individuals for bumble bees), that cooperate in nest building. Thus, a bee colony is a “population of honey bees.”

[0057] Typically, a “honey bee colony” peaks from late spring to summer and reaches a low point in winter. The social structure of the colony is maintained by the queen and workers and depends on an effective system of communication. Domesticated honeybees are cultivated in “beehives” or “honey bee hives.” Thus, the term “beehive” or “honey bee hive”

refers to a structure that functions as a habitation for a colony of bees, e.g., a colony of honey bees.

[0058] As used herein, the term “carrier” encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. A carrier can also be a feed that is appropriate for the invertebrate being treated, e.g., a bee, a shrimp, a mite, a mosquito, a tick, for examples. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see Martin (1975) Remington's Pharm. Sci., 15th Ed. (Mack Publ. Co., Easton).

[0059] The term “effective amount” or “an amount effective to” or any grammatically equivalent term or expression refers to the amount that, when administered by any means to a e.g., an adult or larvae, for treating or preventing a viral disease or condition in a single invertebrate or a population of invertebrates such as a bee colony, and is sufficient to effect treatment or prevention of that viral disease in the individual invertebrate or the population (e.g., bee colony). Typically, an effective dose of antigen for treating and/or immunizing an adult and her respective brood is about 1.5×10^7 or $1.5 \times 10^4 - 1.5 \times 10^{11}$ antigen per dose/gram of food and ranges therebetween. In one embodiment, an "effective amount" refers to that amount of a composition which when fed to an adult is sufficient to vaccinate the adult and the larvae she produces such that larvae from the vaccinated adult are at least 50%, or at least 45%, or at least 40%, or at least 35%, or at least 30%, or at least 25%, or at least 20%, or at least 15 %, or alternatively at least 10%, or at least 5%, more resistant against viral infection than are larvae from an unvaccinated adult.

[0060] The term “nurse bee” as used herein intends are the bees, i.e., worker bees, that feed the worker larvae worker jelly which is secreted from glands that produce royal jelly.

[0061] The term “worker bee” as used herein intends any female (eusocial) bee that lacks the full reproductive capacity of the colony's queen bee.

[0062] The term “brood” intends the three developmental stages in bees, which are collectively known as brood. Bees begin in eggs, which hatch to become larvae (plural) and then undergo metamorphosis as pupae. The larvae is legless and is specialized to eat.

[0063] The term “prophylactic” refers to an agent that acts to prevent a disease e.g., a viral disease.

[0064] The term “vaccinate” as used herein, refers to means for producing immunity against a viral disease e.g., DWV-B and those listed in **Table 1**, or alternatively **Tables 1-4** and **Experiment No. 4**, infra., so as to treat or prevent a disease or condition from occurring (prophylactic treatment) or inhibiting the disease from spreading (slowing or arresting its development) in the brood, larvae, progeny, population, or colony.

[0065] The term “genus” as used herein has its customary meaning as known in the art. In general, genus is defined as taxonomic rank used in the biological classification of living organisms, in the hierarchy of biological classification, genus comes above species and below family. By way of example, the *Paenibacillus* genus is of facultative anaerobic, endospore-forming bacteria, classified by Ash et al. 1994 (see e.g., Ash, C., Priest, F. G. & Collins, M.D. (1994). *Paenibacillus* gen. nov. and *Paenibacillus polymyxa* comb. nov. In Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB, List no. 51. Int J Syst Bacteriol 44, 852). Disease pathogen is an infective biological agent, that causes an illness in a host, characterized by certain features such disturbing the function of an organism, and seriously impairs host, including its demise. Broad spectrum generalized protection is achieved when immunization with a bacterium of specific genus or multiple species of the genus protects against infection of the host then encountering a disease causing pathogen that was not included in vaccine preparation.

[0066] The term “treatment” intends to raise an immune response in the adult which is then passed on to her progeny.

[0067] The term “raise an immune response” intends that the vaccine or treatment produces the non-disease causing antigens in the ovaries of the adult to the developing eggs.

[0068] The term “vaccinating bacteria” intends the pathogenic or non-pathogenic bacteria that is the active immunizing or treating bacteria in the vaccine preparation. The vaccinating bacteria will vary with the invertebrate being treated and the viral disease being treated or prevented. In one aspect, the vaccinating bacteria is PL for the treatment and/or prevention of

Deformed Wing Virus-B (DWV-B) in honey or bumble bees and/or honey or bumble bee colonies and White Spot Syndrome Virus (WSSV) in shrimp.

[0069] The terms “vaccine,” “vaccine formulations,” and/or “vaccinating bacteria” intends the dead and/or inactivate whole cell or cell wall fragments (antigenic fragments) of a bacteria shown to treat and/or prevent a microbial (e.g., bacterial, viral, or fungal) infection upon administration to an invertebrate. The bacteria and/or fragments thereof may be the sole active agent in the vaccine, or it may be combined with other active agents. It should be understood, without explicit recitation that cell wall fragments can be used with or as an alternative to the bacterial whole cells.

[0070] An “antigen/unit” intends the number of cells or antigenic fragments of the non-disease pathogen.

[0071] The term “administers” or “administering” intends any appropriate means to provide the bacterial vaccine to the invertebrate that is the target of treatment. Non-limiting examples of such include providing the vaccine in the food for the invertebrate, providing the vaccine by injection, or providing the vaccine by dissolving the bacterial vaccine in the surrounding environment such as dissolving or suspending in the holding tank or enclosure which house the invertebrate (e.g., shrimp) and immersing the invertebrate in the dissolved or suspended bacterial vaccine.

[0072] The term “dose” intends the amount of bacterial vaccine provided to the invertebrate (e.g., insect or shrimp) in one injection, submersion or one feeding or unit amount of food.

[0073] *Paenibacillus larvae* is a species of bacterium, found worldwide, which causes American foulbrood, a fatal disease of the larvae of honey bees. It is a Gram-positive, rod-shaped bacterium, which forms spores which can remain viable for at least thirty-five years. Strains and genotypes are known (see e.g., Appl. Environ. Microbiol. (2005) Nov. 71(11):7551-7555) and commercially available from the American Type Culture Collection (ATCC). For use in the methods of this disclosure, non-limiting examples of the group includes one or more *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericanus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P.*

campinasensis, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvificiens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wymii*, and/or *P. xylanilyticus*.

[0074] As used herein the term *Vibrio* intends a genus of gram-negative bacteria possessing a curved-rod (comma) shape, several species of which can cause foodborne infection or soft-tissue infection called Vibriosis. Infection is commonly associated with eating undercooked seafood. Non-limiting examples of such include one or more of: *V. adaptatus*, *V. aerogenes*, *V. aestivus*, *V. aestuariamus*, *V. agarivorans*, *V. albensis*, *V. alfacensis*, *V. alginolyticus*, *V. anguillarum*, *V. areninigrae*, *V. artabrorum*, *V. atlanticus*, *V. atypicus*, *V. azureus*, *V. brasiliensis*, *V. bubulus*, *V. calviensis*, *V. campbellii*, *V. casei*, *V. chagasii*, *V. cholerae*, *V. cincinnatiensis*, *V. coralliilyticus*, *V. crassostreae*, *V. cyclitrophicus*, *V. diabolicus*, *V. diazotrophicus*, *V. ezurae*, *V. fluvialis*, *V. fortis*, *V. furnissii*, *V. gallicus*, *V. gazogenes*, *V. gigantis*, *V. haliotocoli*, *V. harveyi*, *V. hepatarius*, *V. hippocampi*, *V. hispanicus*, *V. ichthyenterii*, *V. indicus*, *V. kanaloae*, *V. lentus*, *V. litoralis*, *V. logei*, *V. mediterranei*, *V. metschnikovii*, *V. mimicus*, *V. mytili*, *V. natriegens*, *V. navarrensis*, *V. neonatus*, *V. neptunius*, *V. nereis*, *V. nigripulchritudo*, *V. ordalii*, *V. orientalis*, *V. pacinii*, *V. parahaemolyticus*, *V. pectenocida*, *V. pelagius*, *V. penaeocida*, *V. pomeroyi*, *V. ponticus*, *V. proteolyticus*, *V. rotiferianus*, *V. ruber*, *V. rumoiensis*, *V. salmonocida*, *V. scophthalmi*, *V. splendidus*, *V. superstes*, *V. tapetis*, *V. tasmaniensis*, *V. tubiashii*, *V. vulnificus*, *V. wodanis*, and/or *V. xuii* or a combination of two or more thereof.

[0075] Modes for Carrying Out the Disclosure

[0076] Preparation of Bacterial Vaccine Compositions General Methods

[0077] *Preparation of Bacterial Vaccine Compositions*

[0078] This disclosure provides an invertebrate vaccine composition comprising, consisting essentially of, or consisting of at least one, or at least two, or at least three, or at least four or more of dead and/or inactivated whole cell or cell wall fragments of bacteria. In one aspect, provided is an invertebrate vaccine composition comprising, consisting essentially of, or consisting of at least one, or at least two, or at least three, or at least four or more of dead and/or inactivated gram-positive bacteria and/or at least one, or at least two, or at least three, or at least four or more of dead and/or inactivated gram-negative bacteria. In one embodiment, the invertebrate vaccine composition comprises, consists essentially of, or consists of dead and/or inactivated whole cell or cell wall fragments of the gram-positive bacteria and/or gram-negative bacteria. In another embodiment, the gram-positive bacteria and/or gram-negative bacteria is selected from any one or more of the vaccinating bacteria listed in any one of **Tables 1-4** and/or **Experiment No. 4**. As an example only, the vaccine composition can comprise, or consist essentially of, or yet further consist of dead and/or inactivated whole cell or cell wall fragments of *P. larvae* and *P. thiaminolyticus* for the vaccination of black soldier flies against *Hermetia illucens totiviurs*. The ratios of the individual components of the vaccine can vary, e.g., 1:1; 1:2; 1:3; 1:4; 1:1:4; 1:2:4; 1:3:4. In one aspect, the ratio is 1:1.

[0079] A bacterial pathogen is selected for the vaccine composition and will vary with the infectious disease (e.g., viral disease) and the invertebrate being treated (see for example, **Table 1**, or alternatively **Tables 1-4** and/or **Experiment No. 4**).

[0080] Seed cultures of the selected bacteria are inoculated, cultured, and expanded if necessary. For example, the bacteria can be expanded from frozen glycerol stocks of bacteria and the inoculated plates are grown at the appropriate temperature and conditions for the bacteria, e.g., at 35°C in a darkened growth chamber for 4-12 days.

[0081] The bacterial colonies are harvested for example, by washing the plates with 5 mL ice- cold H₂O and scraping them off into a falcon tube (or glass bottle). The optical density at 600 nm (OD₆₀₀) is measured using standard methods. Equal volumes of harvested bacteria culture and H₂O are mixed in two replicates into cuvettes. Based on the OD₆₀₀ reading,

antigen solutions are prepared to the desired concentration, for example at least 1.5×10^9 bacterial cells/mL. Bacterial solution is inactivated. Non-limiting examples for the inactivation of the collected bacteria include the use of formalin, binary ethylenimine (BEI) or heat and pressure (autoclaving). Any method known in the art for killing bacteria may be used. Following killing, the cells are lysed and large fragments of the bacterial cell walls are separated, for example by centrifugation for between 1-4 hours (or longer, depending on the volume, size and concentration) at a speed from about 10,000g – to about 60,000g. The supernatant is recovered and can be further fractionated as noted below. Alternatively whole cell preparations can be used. The supernatant can be freeze dried, lyophilized or in solution and can be administered to the selected invertebrate in different forms, such as e.g., feed, spray, injection, or immersion. One mode of administration, includes but is not limited to a preparation of a feed selected for the invertebrate, which can be purchased prepacked at supply stores or prepared by methods known in the art. As an example only, the invertebrate feed may be coated with the bacterial vaccine for administration to the invertebrate.

[0082] Administration dose can be determined using standard methods for such as pre-inactivation cell count, or ELISA based potency testing in the bacteria. See, e.g., pdf.sciencedirectassets.com/278679/1-s2.0-S1877282X11X00036/1-s2.0-S1877282X11000348/main.pdf?X-Amz-SecurityToken=IQoJb3JpZ2luX2VjEHsaCXVzLWVhc3QtMSJGMEQCIFHI0kqWKpEoBlN9Mhcg0MVIOXd2ncxY5xEL6FKYb%2FfOAiBTCdnHlABBoPZKjzbYY4yLT4i6kuMTt63YCYqFPzCjS, last accessed December 18, 2024.

[0083] The compositions can be formulated to provide vaccine compositions to provide a total dose of dead and/or inactivated bacteria or cell wall fragments thereof contains between about 1.5×10^4 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 or 1.5×10^5 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 or 1.5×10^6 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{10} antigen units, or from about 1.5×10^8 to about 1.5×10^9 antigen units, or at least 1.5×10^7 or 1.5×10^4 antigen units, or at least 1.5×10^5 antigen units, or at least 1.5×10^6 antigen units, or at least 1.5×10^7 antigen units, or at least about 1.5×10^8 antigen units, or at least about 1.5×10^9 antigen units, or at

least about 1.5×10^{10} antigen units, of the least one dead non-disease species of bacteria or fragments thereof.

[0084] *Preparation of Bacterial Fragments to be Used as Vaccine Antigen*

[0085] **FIG. 1** graphically illustrates an exemplary method to obtain bacterial fragments for use as antigens. BugBuster® is used on fresh or frozen cell pellets. Cells are harvested from liquid culture by centrifugation at $10,000 \times g$ for 10 min using a weighed centrifuge tube. For small scale extractions (1.5 ml or less), centrifugation can be performed in a 1.5-ml tube at $14,000\text{--}16,000 \times g$. Liquid is decanted, and the pellet is allowed to drain, removing as much liquid as possible. The pellet can be weighed. Once the cells are harvested, but not inactivated by for example, use of an autoclave or methods described above, they are pelleted and stored at $+4^{\circ}\text{C}$. The cell pellet is resuspended at room temperature with BugBuster® Master Mix by pipetting or gentle vertexing, using 5 ml reagent per gram of wet cell paste. This typically corresponds to about 2.5 ml per 50-ml culture. The cell suspension is incubated on a shaking platform or rotating mixer at a slow setting for 10–20 min at room temperature, incubated for 1h, shaking, at $+25^{\circ}\text{C}$, and insoluble cell debris is removed by centrifugation at $16,000 \times g$ for 20 min at 4°C . The supernatant is transferred to a fresh tube. Clarified extracts are maintained on ice for short term storage (2–3 h) or frozen at -20°C until needed. Cell homogenate is transferred into centrifuge tubes. Centrifugation is done according to the below scheme and supernatant is transferred to new centrifuge tube.

[0086] Centrifugation steps used (at 4°C):

- 1) 10 min 800xg
- 2) 10 min 1500xg
- 3) 60 min 10000xg
- 4) 3h 20000xg

[0087] add 500µl PBS to each pellet and vortex.

[0088] Collected fragments can be used as an antigen in the food to vaccinate or treat the invertebrates such as shrimp or bee queens and their progeny.

[0089] The compositions can be formulated to provide vaccine compositions to provide a total dose of dead and/or inactivated bacteria or cell wall fragments thereof contains between about 1.5×10^4 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 or 1.5×10^5 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 or 1.5×10^6 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{10} antigen units, or from about 1.5×10^8 to about 1.5×10^9 antigen units, or at least 1.5×10^7 or 1.5×10^4 antigen units, or at least 1.5×10^5 antigen units, or at least 1.5×10^6 antigen units, or at least 1.5×10^7 antigen units, or at least about 1.5×10^8 antigen units, or at least about 1.5×10^9 antigen units, or at least about 1.5×10^{10} antigen units, of the least one dead non-disease species of bacteria or fragments thereof.

[0090] Using the above noted methods, **Tables 1-4** and/or **Experiment No. 4** identify the vaccinating bacteria antigen (pathogen) that are used alone or in combination with each other in the vaccine compositions for the treatment of the invertebrate group and the pathogen causing disease the vaccine is formulated to treat or prevent.

[0091] **Table 1**

Invertebrate Group For Vaccination	Genus	Common Name	Vaccinating Bacteria	Pathogen Causing Disease
Hymenopterans	<i>Apis</i>	honey bee	<i>Paenibacillus larvae</i>	<i>Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus</i>
Hymenopterans	Bombus	bumble bee	<i>Paenibacillus larvae</i>	<i>Deformed Wing Virus A, Deformed Wing</i>

				<i>Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus</i>
Diptera	<i>Hermetia</i>	Black soldier fly	<i>Paenibacillus Thiaminolyticus</i>	Hermetia illucens Totivirus
Coleoptera	<i>Tenebrio</i>	Mealworm beetle	<i>Serratia marcescens</i>	Flacherie, Grasserie
Coleoptera	<i>Alphitobius</i>	Lesser mealworm beetle	<i>Serratia marcescens</i>	Flacherie, Grasserie
Orthopteran	<i>Acheta</i>	house cricket	Rickettsiella, Pseudomonas	Acheta domesticus denisovirus, iridioviruses, Iflaviruses
Orthopteran	<i>Gryllus</i>	field crickets	Rickettsiella, Pseudomonas	Acheta domesticus denisovirus, iridioviruses, Iflaviruses
Orthopteran	<i>Gryllodes</i>	tropical cricket	Rickettsiella, pseudomonas	Acheta domesticus denisovirus, iridioviruses,
Lepidoptera	<i>Bombyx Mori</i>	silk moth	<i>Bacillus thuringiensis (bacterial toxicosis)</i>	Bombyx Mori nuclear polyhedrosis virus, Bombyx Mori cytoplasmic polyhedrosis virus, Bombyx Mori infectus flacherie virus, Bombyx Mori denisonulceosis virus, Bombyx Mori densovirus, Bombyx Mori cypovirus 1
	Rock Shrimp Pink Shrimp	Shrimp	<i>Vibrio sp.</i>	White spot Virus, Yellow-head virus, Taura Syndrome Virus,

	Tiger Shrimp Chinese White Shrimp Brown Shrimp White Shrimp Atlantic Northern Shrimp Spot Shrimp Aesop Shrimp Banana Prawn Blue Shrimp			Infectious Myonecrosis Virus, Infectious hypodermal and haematopoeitic necrosis virus
Shrimp			Paenibacillus sp.or Paenibacillys sp. And Vbrio sp.	White spot Virus, Yellow-head virus, Taura Syndrome Virus, Infectious Myonecrosis Virus, Infectious hypodermal and haematopoeitic necrosis virus <i>Vibrio harveyi, Vibrio alginolyticus, Vibrio anguillarum, Vibrio splendidus, Vibrio salmonicida, Vibrio vulnificus, Vibrio parahaemolyticus Hepatobacter penaeiis</i>

[0092] **Table 2 – Vector Based Diseases**

[0093] **Table 2** provides additional vector-based diseases as examples where the provided bacterial vaccines are used for the reduction of pathogen load in insects (i.e., vectors) which further serve as methods for preventing, treating or immunizing against the transmission of infectious diseases to plants, animals, and/or humans. For the purpose of the diseases listed in

Table 1, the method administers an effective amount of a non-disease causing dead and/or inactivated gram-positive bacterial pathogen or a cell wall fragment thereof alone or in combination with a gram negative bacterial pathogen or a cell wall fragment thereof. Administration methods are described herein.

Table 2 – Vector Based Diseases

Vector	Disease	Type of Pathogen
<i>Asian citrus psyllid</i>	Citrus greening	Bacteria
sap-feeding insects, such as the glassy-winged sharpshooter	Pierce's disease	Bacteria
walnut twig beetle	Thousand cankers disease	Fungus
Thrips	Tomato spotted wilt	Virus
Whiteflies	Yellow mosaic disease	Virus
bark beetles	Dutch elm disease	Fungus
	Chestnut blight	Fungus
pollinators	Fire blight	Bacteria
Mosquito	Chikungunya	Virus
	Dengue	Virus
	Rift Valley fever	Virus
	Yellow Fever	Virus
	Zika	Virus
	O'nyong'nyong virus	Virus
	Japanese encephalitis	Virus
	West Nile fever	Virus
Fleas	Plague (transmitted from rats to humans)	Bacteria
Lice	Typhus	Bacteria
	Louse-borne relapsing fever	Bacteria
Sandflies	Sandfly fever (phlebotomus fever)	Virus
Ticks	Crimean-Congo haemorrhagic fever	Virus
	Lyme disease	Bacteria
	Relapsing fever (borreliosis)	Bacteria
	Rickettsial diseases (e.g., spotted fever and Q fever)	Bacteria
	Tick-borne encephalitis	Virus
	Tularaemia	Bacteria

[0095] Table 3 - Viral pathogens of penaeid shrimp - DNA Virus (reproduced from <https://www.biomin.net/species/aquaculture/shrimp-diseases/>, last accessed on December 28, 2023.)

DISEASE	VIRUS
Nimaviridae	White spot syndrome virus (WSSV)
Parvoviridae	Infectious hypodermal and hematopoietic necrosis virus (IHHNV)
Hepatopancreatic parvovirus (HPV)	
Spawner-isolated mortality virus (SMV)	
Lymphoid parvo-like virus (LPV)	
Baculoviridae	Baculovirus penaei (BP)
Monodon baculovirus (MBV)	
Baculovirus midgut gland necrosis virus (BMNV)	
Type C baculovirus of <i>Penaeus monodon</i>	
Iridoviridae	Shrimp iridovirus (IRIDO)
<i>Shrimp</i> hemocyte iridescent virus (SHIV)	

[0096] Table 4 - Viral pathogens of penaeid shrimp - RNA Virus (reproduced from <https://www.biomin.net/species/aquaculture/shrimp-diseases/>, last accessed on December 28, 2023.)

DISEASE	VIRUS
Picornaviridae	Taura syndrome virus (TSV)
Totiviridae	Infectious myonecrosis virus (IMNV)
Nodaviridae	Covert mortality nodavirus (CMNV)
<i>Penaeus vannamei</i> nodavirus (PvNV)	
Roniviridae	Yellow head virus (YHV)
Gill associated virus (GAV)	
Lymphoid organ virus (LOV)	
Reoviridae	Reo-like virus (REO) type II and IV
Togaviridae	Lymphoid organ vacuolization virus (LOVV)
Rhabdoviridae	Rhabdovirus of penaeid shrimp (RPS)

[0097] Preparation of *Paenibacillus* larvae Vaccine and *Paenibacillus* larvae-*Vibrio parahaemolyticus* (VP) Combination Product Compositions

[0098] The following illustrates exemplary methods for preparing a composition comprising an effective amount of one or more species of dead and/or inactivated whole or fragmented *Paenibacillus larvae* and *Vibrio sp* combination product.

[0099] *Paenibacillus larvae* (PL) can be prepared to provide a vaccine or treatment for shrimp.

[0100] To do so, seed cultures are inoculated from frozen glycerol stocks of PL. The inoculated plates are grown at 35°C in a darkened growth chamber for 4-12 days.

[0101] *Paenibacillus larvae* colonies are harvested by washing the plates with 5 mL ice-cold H₂O and scraping them off into a falcon tube (or glass bottle). The optical density at 600 nm (OD₆₀₀) is measured using standard methods. Equal volumes of harvested bacteria culture and H₂O are mixed in two replicates into cuvettes. Based on the OD₆₀₀ reading, antigen solutions are prepared to the desired concentration – at least 1.5×10^9 bacterial cells/mL. Bacterial solution is inactivated. Non-limiting example for the inactivation of the collected bacteria are the use of formalin, binary ethylenimine (BEI) or heat and pressure (autoclaving). Any method known in the art for killing bacteria may be used. Following killing, the cells can be lysed and large fragments of separated by centrifugation for between 1-4 hours (or longer, depending on the volume, size and concentration) at a speed from about 10,000g – to about 60,000g. The supernatant is recovered and can be further fractionated as noted below. Alternatively whole cell preparations can be used.

[0102] The supernatant can be freeze dried, lyophilized or in solution and can be administered to the bees in different forms, such as e.g., feed, spray, injection. One mode of administration, includes but is not limited to a preparation of insect feed, such as the queen bee candy, which can be purchased prepacked at beekeeping supply stores or prepared by methods known in the art. The supernatant can be used in a combination vaccine composition with one or more, two or more, three or more, or four or more dead and/or inactivated whole or fragmented bacterial species.

[0103] For the production of VP, for the methods of Experiment No. 3, VP strain ATCC-27519 was used. Bacteria were cultured in grown in Marine 2216 broth at 37°C ± 2°C incubator at 250 ± 50rpm for one (1) day. The exponentially growing culture was inactivated

by autoclaving at 121°C for 15 minutes. The inactivation was confirmed by plating on Marine 2216 agar plates (10 plates) and incubating at 37°C for 14 days and observed for any growth of visible colonies. Additionally, plating on TSA blood agar plates (2 plates) and incubating at 37°C for 24-48 hours was done and observed for any growth of visible colonies. No growth on all inoculated plates confirmed sterility. Heat inactivated vaccines were stored at 2-8°C until used for vaccination.

[0104] The compositions can be formulated to provide vaccine compositions to provide a total dose of dead and/or inactivated bacteria or cell wall fragments thereof contains between about 1.5×10^4 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 or 1.5×10^5 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 or 1.5×10^6 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{10} antigen units, or from about 1.5×10^8 to about 1.5×10^9 antigen units, or at least 1.5×10^7 or 1.5×10^4 antigen units, or at least 1.5×10^5 antigen units, or at least 1.5×10^6 antigen units, or at least 1.5×10^7 antigen units, or at least about 1.5×10^8 antigen units, or at least about 1.5×10^9 antigen units, or at least about 1.5×10^{10} antigen units, of the least one dead non-disease species of bacteria or fragments thereof.

[0105] Prepared for parameter investigation and viable challenge test. *Vibrio* and WSSV challenge material were prepared by ShrimpVet Laboratory, Vietnam.

[0106] *Feed Premixing with Vaccines or Placebo*

[0107] Commercial shrimp pellet feeds were coated with either the vaccine treatments or placebo. The vaccine treatments at a dose of $> 10^9$ cells /mL were coated to saturate the feed pellet. Placebo (sterile culture medium) treatment was coated onto pellet feeds similarly. Premixed feed treatments were stored at 2-8°C until used for vaccination.

[0108] *Preparation of PL Bacterial Fragments and VP Bacterial Fragments to be Used as Vaccine Antigen*

[0109] **FIG. 1** graphically illustrates an exemplary method to obtain PL and/or VP bacterial fragments to be used as antigens. BugBuster® can be used on fresh or frozen cell pellets. Cells are harvested from liquid culture by centrifugation at $10,000 \times g$ for 10 min using a

weighed centrifuge tube. For small scale extractions (1.5 ml or less), centrifugation can be performed in a 1.5-ml tube at $14,000\text{--}16,000 \times g$. Liquid is decanted and the pellet is allowed to drain, removing as much liquid as possible. Pellet is weighed. Once the cells are harvested, but not autoclaved, they can be pelleted and stored at $+4^{\circ}\text{C}$. The cell pellet is resuspended at room temperature with BugBuster® Master Mix by pipetting or gentle vortexing, using 5 ml reagent per gram of wet cell paste. This typically corresponds to about 2.5 ml per 50-ml culture. The cell suspension is incubated on a shaking platform or rotating mixer at a slow setting for 10–20 min at room temperature. Incubated for 1h, shaking, $+25^{\circ}\text{C}$. Insoluble cell debris is removed by centrifugation at $16,000 \times g$ for 20 min at 4°C . The supernatant is transferred to a fresh tube. Clarified extracts are maintained on ice for short term storage (2–3 h) or frozen at -20°C until needed. Cell homogenate is transferred into centrifuge tubes. Centrifugation is done according to the below scheme and supernatant is transferred to new centrifuge tube.

[0110] Centrifugation steps used (at 4°C):

- 1) 10 min $800\times g$
- 2) 10 min $1500\times g$
- 3) 60 min $10000\times g$
- 4) 3h $20000\times g$

[0111] add 500µl PBS to each pellet and vortex.

[0112] Collected PL fragments, alone or in combination with VP, can be used as an antigen in a composition to vaccinate or treat bees, shrimp, and their progeny.

[0113] The compositions can be formulated to provide vaccine compositions to provide a total dose of dead and/or inactivated bacteria or cell wall fragments thereof contains between about 1.5×10^4 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 or 1.5×10^5 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 or 1.5×10^6 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{10} antigen units, or from about 1.5×10^8 to about 1.5×10^9 antigen units, or at least 1.5×10^7 or 1.5×10^4 antigen units, or at

least 1.5×10^5 antigen units, or at least 1.5×10^6 antigen units, or at least 1.5×10^7 antigen units, or at least about 1.5×10^8 antigen units, or at least about 1.5×10^9 antigen units, or at least about 1.5×10^{10} antigen units, of the least one dead non-disease species of bacteria or fragments thereof.

[0114] In an exemplary embodiment, the composition and/or vaccine preparation is given to worker honey bees that will incorporate the vaccine with royal jelly in the mandibular glands and feed it to the honey bee queen or it is given directly to a honey bee queen to effectively treat or immunize the queen and her resulting brood of larvae against the virus causing the disease, e.g. Deformed Wing Virus-B (DWV-B).

[0115] In another exemplary embodiment, the composition and/or vaccine preparation is given to maternal broodstock to effectively treat and immunize the maternal shrimp and her resulting in her brood of larvae against the bacteria and viruses causing disease, e.g. Vibriosis and White Spot Syndrome Virus Disease (WSSV). The composition and/or vaccine preparation may be administered to the maternal broodstock as a shrimp feed coated with the composition and/or vaccine preparation, as an injection, or the maternal broodstock may be immersed or submerged in a solution or suspension of the composition and/or vaccine preparation.

[0116] For the shrimp feed, commercially-available feed can be used and the vaccine can be combined with the feed by simple mixing or coating of the feed with the vaccine. A non-limiting example of a commercial feed is Skretting SAPPHIRE feed (see here www.skretting.com/en-in/feed-and-services-for-aquaculture/sapphire-4853/) as an example of commercially available feed, also described below.

[0117] *Vaccination Methods*

[0118] *Invertebrate Population Treatment or Prevention*

[0119] This disclosure provides a method for preventing or treating an infection, e.g., viral or microbial infection and/or disease in an invertebrate of a population of invertebrates such as those identified in **Table 1** or alternatively **Tables 1-4** and **Experiment No. 4**, e.g., shrimp, crustaceans, and insects such as bees, e.g., honey bees. In one aspect, the method comprises or consists essentially of, or yet further consists of administering or feeding to the population

of invertebrates who produces or supports the invertebrate progeny or the progeny itself a bacterial vaccine comprising dead and/or inactivated bacteria or fragments thereof specifically selected for the treatment of the invertebrate population and the virus causing the viral disease. In an aspect, the administering comprises, consists essentially of, or consists of oral administration, injection, submersion, or a combination of any two or more thereof.

[0120] In another aspect, the method comprises, or consists essentially of, or consists of feeding an effective amount of a bacterial vaccine composition comprising dead and/or inactivated bacteria or fragments thereof to the adult invertebrate in the population who then passes on the immunity and/or treatment to its progeny in the population. In a further aspect, the progeny in the population is administered the vaccine. In one aspect, the vaccine formulation is contained or mixed with a typical food for the invertebrate which acts as a carrier. As an example only, the food for the invertebrate may be coated with the vaccine formulation. In one aspect, from about 1.5×10^7 or 1.5×10^4 to about 1.5×10^8 antigen units/gram of food is provided to or fed to the invertebrate, such as shrimp, an insect, such as queen bee, worker bees, nurse bees and/or the larvae (see **Table 1** for a list of paired invertebrates, bacterial vaccines and the virus causing the viral infections, **Table 2** for a list of vector-based diseases that may be transmitted from the vector (e.g., insect) to animals, plants, and/or humans, and **Tables 3 and 4** for a list of shrimp viral pathogens causing disease in shrimp, and **Experiment No. 4** for mosquito-borne transmission).

[0121] In one aspect of this method, the administering comprises feeding the invertebrates a suitable amount of food, wherein the amount administered per dose a vaccine, composition or formulation comprising dead and/or inactivated bacteria or fragments thereof contains between about 1.5×10^4 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 or 1.5×10^5 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 or 1.5×10^6 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{10} antigen units, or from about 1.5×10^8 to about 1.5×10^9 antigen units, or at least 1.5×10^4 antigen units, or at least 1.5×10^5 antigen units, or at least 1.5×10^6 antigen units, or at least 1.5×10^7 antigen units, or at least about 1.5×10^8 antigen units, or at least about 1.5×10^9 antigen units, or at

least about 1.5×10^{10} antigen units, of the least one dead non-disease species of bacteria or fragments thereof.

[0122] In a further aspect, the method further comprises testing (and such methods are known in the art) the invertebrate population for the microbial infection prior to administration of the vaccine. In a yet further aspect, the bacterial vaccine is selected to specifically treat and/or prevent the viral infection and the invertebrate population and an effective amount of the vaccine is then administered to the invertebrate population (see **Table 1** for a list of paired invertebrates, bacterial vaccines and viral infections treated by the bacterial vaccines, **Table 2** for a list of vector-based diseases that may be transmitted from the vector (e.g., insect) to animals, plants, and/or humans, and **Tables 3** and **4** for a list of shrimp viral pathogens and the viral diseases).

[0123] In a further aspect, for the treatment of infected population, an infected or uninfected adult who produces progeny is administered the vaccine formulation and introduced into the population and the vaccine formulation is then passed on to the progeny.

[0124] *Vaccination Methods: Invertebrate and Invertebrate Population Bacterial or Microbial Treatment or Prevention*

[0125] This disclosure provides a method for preventing or treating a microbial infection and/or disease in a population of invertebrates or an invertebrate such as those identified in **Table 1**, or alternatively **Tables 1-4** and **Experiment No. 4**, e.g., shrimp, crustaceans, and insects such as bees, e.g., honey bees. In one aspect, the method comprises or consists essentially of, or yet further consists of administering or feeding to the population of invertebrates who produces or supports the invertebrate progeny or the progeny itself a bacterial vaccine comprising dead and/or inactivated bacteria or fragments thereof specifically selected for the treatment of the invertebrate population and the virus causing the viral disease. In an aspect, the administering comprises, consists essentially of, or consists of oral administration, injection, submersion, or a combination of any two or more thereof.

[0126] In another aspect, the method comprises, or consists essentially of, or consists of feeding an effective amount of a bacterial vaccine composition comprising dead and/or inactivated bacteria or fragments thereof to the adult invertebrate in the population who then

passes on the immunity and/or treatment to its progeny in the population. In a further aspect, the progeny in the population is administered the vaccine. In one aspect, the vaccine formulation is contained or mixed with a typical food for the invertebrate which acts as a carrier. As an example only, the food for the invertebrate may be coated with the vaccine formulation. In one aspect, from about 1.5×10^7 or 1.5×10^4 to about 1.5×10^8 antigen units/gram of food is provided to or fed to the invertebrate, such as shrimp, an insect, such as queen bee, worker bees, nurse bees and/or the larvae (see **Table 1** for a list of paired invertebrates, bacterial vaccines and the virus causing the viral infections, **Table 2** for a list of vector-based diseases that may be transmitted from the vector (e.g., insect) to animals, plants, and/or humans, and **Tables 3 and 4** for a list of shrimp viral pathogens causing disease in shrimp) and **Experiment No. 4** for mosquito-borne diseases.

[0127] In one aspect of this method, the administering comprises feeding the invertebrates a suitable amount of food, wherein the amount administered per dose a vaccine, composition or formulation comprising dead and/or inactivated bacteria or fragments thereof contains between about 1.5×10^4 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 or 1.5×10^5 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 or 1.5×10^6 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{10} antigen units, or from about 1.5×10^8 to about 1.5×10^9 antigen units, or at least 1.5×10^4 antigen units, or at least 1.5×10^5 antigen units, or at least 1.5×10^6 antigen units, or at least 1.5×10^7 antigen units, or at least about 1.5×10^8 antigen units, or at least about 1.5×10^9 antigen units, or at least about 1.5×10^{10} antigen units, of the least one dead non-disease species of bacteria or fragments thereof.

[0128] In a further aspect, the method further comprises testing (and such methods are known in the art) the invertebrate population for the microbial infection prior to administration of the vaccine. In a yet further aspect, the bacterial vaccine is selected to specifically treat and/or prevent the viral infection and the invertebrate population and an effective amount of the vaccine is then administered to the invertebrate population (see **Table 1** for a list of paired invertebrates, bacterial vaccines and viral infections treated by the bacterial vaccines, **Table 2** for a list of vector-based diseases that may be transmitted from the vector (e.g., insect) to

animals, plants, and/or humans, and **Tables 3 and 4** for a list of shrimp viral pathogens and the viral diseases and **Experiment No. 4** for mosquito-borne disease and transmission).

[0129] In a further aspect, for the treatment of infected population, an infected or uninfected adult who produces progeny is administered the vaccine formulation and introduced into the population and the vaccine formulation is then passed on to the progeny.

[0130] *Treatment or Prevention of Microbial and Viral Infections in Bees and Shrimp*

[0131] *Honey Bees*

[0132] This disclosure provides a method for preventing or treating microbial infections and/or diseases in a bee, for example wherein the bee is a honey bee and wherein a virus causing the viral infection is selected from the group of Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus. The method comprises or consists essentially of, or yet further consist of administering or feeding to the honey bee who produces or supports honey bee progeny or the progeny itself a bacterial vaccine comprising a dead and/or inactivated gram-positive bacteria or cell wall fragments thereof as described herein. In one aspect, the method comprises, or consists essentially of, or consists of feeding or injecting an effective amount of the gram-positive bacterial vaccine composition to the honey bee queen who then passes on the immunity and/or treatment to its progeny. In a further aspect, the progeny is administered the gram-positive bacterial vaccine. In one aspect, the gram-positive bacterial vaccine formulation is contained or mixed with a typical food for the honey bee queen, honey bee worker bees, honey bee nurse bees or the honey bee larvae, the food acting as a carrier. In one aspect, from about 1.5×10^7 or 1.5×10^4 to about 1.5×10^8 antigen units/gram of food is provided to or fed to the honey bee queen, honey bee worker bees, honey bee nurse bees or the honey bee larvae.

[0133] In one aspect of this method, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated bacterial species of the genus *Paenibacillus*. In another aspect, the dead and/or inactivated bacterial species is selected

from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericanus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvificiens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynmii*, and/or *P. xylanilyticus* or a combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvens*, or a combination of two or more thereof. In yet another aspect, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof.

[0134] In one aspect of this method, the administering comprises feeding the honey bee a suitable amount of food, wherein the amount administered per dose a vaccine, composition or formulation comprising dead and/or inactivated gram-positive bacteria, e.g., PL contains between about 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^5 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^6 to

about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^7 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^{10} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^9 antigen units/gram of food, or at least 1.5×10^7 or 1.5×10^4 antigen units/gram of food, or at least 1.5×10^5 antigen units/gram of food, or at least 1.5×10^6 antigen units/gram of food, or at least 1.5×10^7 antigen units/gram of food, or at least about 1.5×10^8 antigen units/gram of food, or at least about 1.5×10^9 antigen units/gram of food, or at least about 1.5×10^{10} antigen units/gram of food, of the least one dead and/or inactivated gram-positive bacteria or cell wall fragments thereof.

[0135] In one embodiment, but not limited to the composition of oral vaccine is delivered as part of the normal husbandry practice for queen bees. The queens together with 8-10 worker bees are placed into 'queen cages' supplied with sufficient feed (queen candy) to last up to one or two weeks. The oral vaccine is added to the queen candy, on which the queen bee and the worker bees will be feeding for 3-8 days after which she is placed into the new hive, ready to lay eggs and create the new protected bee colony.

[0136] In another embodiment the vaccine is placed into the queen candy in the queen shipping box or into the nuc or queen rearing hive where nurse bees will consume the vaccine with the queen candy and transport it to their royal jelly glands where it is mixed with royal jelly that is fed to the queen bee larvae or the queen bee.

[0137] In another embodiment the vaccine is fed to the developing queen larvae during the larval rearing phase in the queen rearing hives.

[0138] This disclosure provides a method for preventing or treating viral infection and/or disease in a bee, for example wherein the bee is a honey bee and wherein the virus causing the viral infection is Deformed Wing Virus B (DWV-B). The method comprises or consists essentially of, or yet further consist of administering or feeding to the honey bee who produces or supports honey bee progeny or the progeny itself a bacterial vaccine comprising dead and/or inactivated *P. larvae* as described herein. In one aspect, the method comprises, or consists essentially of, or consists of feeding an effective amount of dead and/or inactivated *P. larvae* bacterial vaccine composition to the honey bee queen who then passes on the

immunity and/or treatment to its progeny. In a further aspect, the progeny is administered the dead and/or inactivated *P. larvae* vaccine. In one aspect, the dead and/or inactivated PL vaccine formulation is contained or mixed with a typical food for the honey bee queen, honey bee worker bees, honey bee nurse bees or the honey bee larvae, the food acting as a carrier. In one aspect, from about 1.5×10^4 to about 1.5×10^8 antigen units/gram of food is provided to or fed to the honey bee queen, honey bee worker bees, honey bee nurse bees or the honey bee larvae.

[0139] In one aspect of this method to treat or prevent DWV-B, the administering comprises feeding the honey bee a suitable amount of food, wherein the amount administered per dose a vaccine, composition or formulation comprising dead and/or inactivated PL or cell wall fragments thereof contains between about 1.5×10^4 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^7 or 1.5×10^5 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^6 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^7 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^{10} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^9 antigen units/gram of food, or at least 1.5×10^4 antigen units/gram of food, or at least 1.5×10^5 antigen units/gram of food, or at least 1.5×10^6 antigen units/gram of food, or at least 1.5×10^7 antigen units/gram of food, or at least about 1.5×10^8 antigen units/gram of food, or at least about 1.5×10^9 antigen units/gram of food, or at least about 1.5×10^{10} antigen units/gram of food, of the least one dead and/or inactivated PL or cell wall fragments thereof.

[0140] In one embodiment, but not limited to the composition of oral vaccine is delivered as part of the normal husbandry practice for queen bees. The queens together with 8-10 worker bees are placed into 'queen cages' supplied with sufficient feed (queen candy) to last up to one or two weeks. The oral vaccine is added to the queen candy, on which the queen bee and the worker bees will be feeding for 3-8 days after which she is placed into the new hive, ready to lay eggs and create the new protected bee colony.

[0141] In another embodiment the vaccine is placed into the queen candy in the queen shipping box or into the nuc or queen rearing hive where nurse bees will consume the vaccine

with the queen candy and transport it to their royal jelly glands where it is mixed with royal jelly that is fed to the queen bee larvae or the queen bee.

[0142] In another embodiment the vaccine is fed to the developing queen larvae during the larval rearing phase in the queen rearing hives.

[0143] *Bumble Bees*

[0144] This disclosure also provides a method for preventing or treating microbial infections and/or diseases in a bee, for example wherein the bee is a bumble bee and wherein the virus causing the viral infection is selected from the group of Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus. The method comprises or consists essentially of, or yet further consist of administering or feeding to the bumble bee who produces or supports bumble bee progeny or the progeny itself a bacterial vaccine comprising dead and/or inactivated gram-positive bacteria or fragments thereof as described herein. In one aspect, the method comprises, or consists essentially of, or consists of feeding an effective amount of the dead and/or inactivated gram-positive bacterial vaccine composition to the bumble bee queen who then passes on the immunity and/or treatment to its progeny.

[0145] In one aspect of this, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated bacterial species of the genus *Paenibacillus*. In another aspect, the dead and/or inactivated bacterial species is selected from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericamus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasilensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jambilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P.*

macquariensis, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvifaciens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynnii*, and/or *P. xylanilyticus* or a combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvans*, or a combination of two or more thereof. In yet another aspect, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof.

[0146] In a further aspect, the progeny is administered the dead and/or inactivated gram-positive bacterial vaccine. In one aspect, the dead and/or inactivated gram-positive bacterial vaccine formulation is contained or mixed with a typical food for the bumble bee queen, bumble bee worker bees, bumble bee nurse bees or the bumble bee larvae, the food acting as a carrier. In one aspect, from about 1.5×10^4 to about 1.5×10^8 antigen units/gram of food is provided to or fed to the bumble bee queen, bumble bee worker bees, bumble bee nurse bees or the bumble bee larvae.

[0147] In one aspect of this method, administration comprises feeding the bumble bee a suitable amount of food, wherein the amount administered per dose a vaccine, composition or formulation comprising dead and/or inactivated gram-positive bacteria or cell wall fragments thereof contains between about 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^5 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^6 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^7 to about 1.5

$\times 10^{11}$ antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^{10} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^9 antigen units/gram of food, or at least 1.5×10^4 antigen units/gram of food, or at least 1.5×10^5 antigen units/gram of food, or at least 1.5×10^6 antigen units/gram of food, or at least 1.5×10^7 antigen units/gram of food, or at least about 1.5×10^8 antigen units/gram of food, or at least about 1.5×10^9 antigen units/gram of food, or at least about 1.5×10^{10} antigen units/gram of food, of the least one dead non-disease PL or fragments thereof.

[0148] In one embodiment, but not limited to the composition of oral vaccine is delivered as part of the normal husbandry practice for queen bees. The queens together with 8-10 worker bees are placed into 'queen cages' supplied with sufficient feed (queen candy) to last up to one or two weeks. The oral vaccine is added to the queen candy, on which the queen bee and the worker bees will be feeding for 3-8 days after which she is placed into the new hive, ready to lay eggs and create the new protected bee colony.

[0149] In another embodiment the vaccine is placed into the queen candy in the queen shipping box or into the nuc or queen rearing hive where nurse bees will consume the vaccine with the queen candy and transport it to their royal jelly glands where it is mixed with royal jelly that is fed to the queen bee larvae or the queen bee.

[0150] In another embodiment the vaccine is fed to the developing queen larvae during the larval rearing phase in the queen rearing hives.

[0151] In a further aspect, the above methods further comprise testing (and such methods are known in the art) the honey bee or bumble bee for the virus or viral infection prior to administration of the dead and/or inactivated PL vaccine and the PL vaccine is administered to the honey bee or bumble bee, e.g., bee queen, bee worker bee, bee nurse bee or the bee larvae.

[0152] *Shrimp*

[0153] *White Spot Syndrome Virus (WSSV)*

[0154] White spot syndrome virus (WSSV) is by far the most devastating pathogen of farmed shrimp. It infects all cultured penaeids and has been responsible for much of the economic impact of disease on shrimp production globally.

[0155] The White Spot Syndrome Virus (WSSV) is a highly virulent pathogen affecting cultured shrimp. It can cause up to 100% mortality within 3–10 days. WSSV is a large, enveloped double-stranded DNA virus belonging to the *Whispovirus* genus in the *Nimaviridae* family. It has a broad host range among crustaceans (e.g., shrimp, crabs, lobsters, and crayfish) and primarily impacts commercially cultivated marine shrimp species. The virus infects all age groups, leading to significant mortalities. Tissues of ectodermal and mesodermal origin, such as gills, lymphoid organs, and cuticular epithelium, are the main sites of infection. White spot disease caused by WSSV remains a significant obstacle to sustainable shrimp farming.

[0156] White Spot Syndrome Virus (WSSV) has a broad host range, affecting cultured and wild marine shrimps, crabs, lobsters, crayfish, squilla, copepods, and freshwater species like *Macrobrachium rosenbergii*. It poses a potential threat to most commercially important penaeid shrimp species, including *P. monodon*, *P. vannamei*, *P. indicus*, *P. japonicus*, *P. chinensis*, *P. penicillatus*, *P. azteus*, *P. merguiensis*, *F. duorarum*, and *P. stylirostris*.

[0157] Acute hepatopancreatic necrosis disease (AHPND)

[0158] Vibriosis, an important bacterial disease, caused by opportunistic *Vibrio* spp. continues as the most serious threat to shrimp farmers. *V. harveyi*, *V. alginolyticus*, *V. anguillarum*, *V. splendidus*, *V. salmonicida*, *V. vulnificus* and *V. parahaemolyticus* strains have been found as the main causative organisms of vibriosis. Acute hepatopancreatic necrosis disease (AHPND), initially referred to as early mortality syndrome (EMS), is a relatively recent bacterial ailment affecting farmed penaeid shrimp. Acute hepatopancreatic necrosis disease (AHPND) results from infection with *Vibrio parahaemolyticus* strains (*VpAHPND*) carrying a ~70-kbp plasmid containing genes encoding *Photobacterium* insect-related (Pir) toxins, specifically PirA and PirB. While other *Vibrio* species have been isolated from clinical AHPND cases, only *VpAHPND* has been confirmed as the causative agent.

[0159] AHPND affects various shrimp species, including commercially important ones like *P. monodon*, *L. vannamei*, and *M. rosenbergii*, as well as the brine shrimp, *Artemia franciscana*. Shrimp in their early life stages are particularly susceptible to AHPND infection. AHPND is characterized by high mortality generally when shrimp is 1 month old or post larvae is around 20–30 days old. The disease is characterized by severe hepatopancreatic atrophy, unique histopathological changes during the acute stage, and subsequent massive sloughing of hepatopancreatic or digestive tract epithelial cells within approximately the first 30 days of shrimp post-larvae stocking. Notably, AHPND-causing bacteria primarily target the digestive gland (hepatopancreas), damaging hepatopancreatic R (resorptive), B (blister), F (fibrillar), and E (embryonic) cells, leading to dysfunction and significant shrimp mortalities. Affected shrimp exhibit lethargy, anorexia, slow growth, an empty digestive tract, and a pale to white hepatopancreas.

[0160] Traditional approaches, such as antibiotics and disinfectants, have shown limited effectiveness in mitigating or curing AHPND. Furthermore, their use has been linked to changes in host gut microbiota, immunity, and the development of antibiotic resistance in bacterial pathogens. For instance, the Mexico AHPND-causing *V. parahaemolyticus* strain (13-306D/4 and 13-511/A1) carries the *tetB* gene responsible for tetracycline resistance, while *V. campbellii* from China harbors multiple antibiotic resistance genes. Consequently, there is an urgent need to develop innovative health management strategies such as vaccination to prevent or control AHPND in shrimp aquaculture. This effort is crucial for ensuring future food security and providing economic stability to farmers.

Crustacean and Shrimp Vaccine Compositions and Vaccination Methods

[0161] This disclosure provides a method for preventing or treating microbial infections and/or diseases in a crustacean such as a shrimp, for example wherein the shrimp is selected from the group of Rock Shrimp, Pink Shrimp, Tiger Shrimp, Chinese White Shrimp, Brown Shrimp, White Shrimp, Atlantic Northern Shrimp, Spot Shrimp, Aesop Shrimp, Banana Prawn, and Blue Shrimp. In one aspect of this method, the pathogen causing the microbial infection is selected from the group of White spot syndrome virus (WSSV), Infectious hypodermal and hematopoietic necrosis virus (IHHNV), Baculovirus penaei (BP), Shrimp iridovirus (IRIDO), Taura syndrome virus (TSV), Infectious myonecrosis virus (IMNV), or

Covert mortality nodavirus (CMNV), or those identified in **Tables 3 and 4**, *infra*. In a further aspect, the bacterial vaccine comprises dead and/or inactivated whole cell or cell wall fragments of a gram-positive bacteria and/or a gram-negative bacteria. In a further aspect, the shrimp or population thereof to be vaccinated does not include *P. polymyxa* for the vaccine or feed against *V. parahaemolyticus* infections or disease, when *P. polymyxa* is the sole active agent in the vaccine. In another aspect, the vaccine for treatment of shrimp, the colony or offspring does not include *V. anguillarum* for the treatment or prevention of infection caused by a *Vibrio* species in shrimp.

[0162] In one aspect of this method, the dead and/or inactivated whole cell or cell wall fragments of the gram-negative bacteria comprises a dead and/or inactivated bacterial species of the genus *Vibrio*. In a further aspect, the gram-negative bacteria is selected from *V. adaptatus*, *V. aerogenes*, *V. aestivus*, *V. aestuariamus*, *V. agarivorans*, *V. albensis*, *V. alfacensis*, *V. alginolyticus*, *V. anguillarum*, *V. areninigræ*, *V. artabrorum*, *V. atlanticus*, *V. atypicus*, *V. azureus*, *V. brasiliensis*, *V. bubulus*, *V. calviensis*, *V. campbellii*, *V. casei*, *V. chagasii*, *V. cholerae*, *V. cincinnatiensis*, *V. coralliilyticus*, *V. crassostreae*, *V. cyclitrophicus*, *V. diabolicus*, *V. diazotrophicus*, *V. ezurae*, *V. fluvialis*, *V. fortis*, *V. furnissii*, *V. gallicus*, *V. gazogenes*, *V. gigantis*, *V. haliotocoli*, *V. harveyi*, *V. hepatarius*, *V. hippocampi*, *V. hispanicus*, *V. ichthyenteri*, *V. indicus*, *V. kanaloae*, *V. lentus*, *V. litoralis*, *V. logei*, *V. mediterranei*, *V. metschnikovii*, *V. mimicus*, *V. mytili*, *V. natriegens*, *V. navarrensis*, *V. neonatus*, *V. neptunius*, *V. nereis*, *V. nigripulchritudo*, *V. ordalii*, *V. orientalis*, *V. pacinii*, *V. parahaemolyticus*, *V. pectenica*, *V. pelagius*, *V. penaeicida*, *V. pomeroyi*, *V. ponticus*, *V. proteolyticus*, *V. rotiferianus*, *V. ruber*, *V. rumoiensis*, *V. salmonicida*, *V. scopthalmi*, *V. splendidus*, *V. superstes*, *V. tapetis*, *V. tasmaniensis*, *V. tubiashii*, *V. vulnificus*, *V. wodanis*, and/or *V. xuii* or a combination of two or more thereof, or alternatively selected from the group consisting of *Vibrio alginolyticus*, *V. anguillarum*, *V. campbelli*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*, *V. penaeicida*, *V. vulnificus*, *V. nereis*, *V. tubiashi*, *V. fluvialis*, *V. splendidus*, and/or *V. nigripulchritudo* or a combination of two or more thereof.

[0163] In another aspect of this method, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated bacterial species of the genus *Paenibacillus*. In another aspect, the dead and/or inactivated bacterial species is selected

from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericamus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvificiens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynmii*, and/or *P. xylanilyticus* or a combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvans*, or a combination of two or more thereof. In yet another aspect, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof. In a further aspect, the bacterial vaccine comprises dead and/or inactivated bacterial species of the genus *Paenibacillus* and/or dead and/or inactivated bacterial species of the genus *Vibrio*.

[0164] In one aspect of this method, the method comprises or consists essentially of, or yet further consist of administering to the shrimp who produces or supports shrimp progeny or the progeny itself a bacterial vaccine comprising dead and/or inactivated of a gram-positive

bacteria and/or a gram-negative bacteria whole cells or cell wall fragments thereof. In a further aspect of this method, the bacterial vaccine comprises dead and/or inactivated whole cell or cell wall fragments of *V. adaptatus*, *V. aerogenes*, *V. aestivus*, *V. aestuariamus*, *V. agarivorans*, *V. albensis*, *V. alfacensis*, *V. alginolyticus*, *V. anguillarum*, *V. areninigræ*, *V. artabrorum*, *V. atlanticus*, *V. atypicus*, *V. azureus*, *V. brasiliensis*, *V. bubulus*, *V. calviensis*, *V. campbellii*, *V. casei*, *V. chagasii*, *V. cholerae*, *V. cincinnatiensis*, *V. coralliilyticus*, *V. crassostreae*, *V. cyclitrophicus*, *V. diabolicus*, *V. diazotrophicus*, *V. ezurae*, *V. fluvialis*, *V. fortis*, *V. furnissii*, *V. gallicus*, *V. gazogenes*, *V. gigantis*, *V. haliotocoli*, *V. harveyi*, *V. hepatarius*, *V. hippocampi*, *V. hispanicus*, *V. ichthyenteri*, *V. indicus*, *V. kanaloae*, *V. lentus*, *V. litoralis*, *V. logei*, *V. mediterranei*, *V. metschnikovii*, *V. mimicus*, *V. mytili*, *V. natriegens*, *V. navarrensis*, *V. neonatus*, *V. neptunius*, *V. nereis*, *V. nigripulchritudo*, *V. ordalii*, *V. orientalis*, *V. pacinii*, *V. parahaemolyticus*, *V. pectenida*, *V. pelagius*, *V. penaeicida*, *V. pomeroyi*, *V. ponticus*, *V. proteolyticus*, *V. rotiferianus*, *V. ruber*, *V. rumoiensis*, *V. salmonicida*, *V. scophthalmi*, *V. splendidus*, *V. superstes*, *V. tapetis*, *V. tasmaniensis*, *V. tubiashii*, *V. vulnificus*, *V. wodanis*, and/or *V. xuii* or a combination of two or more thereof, or alternatively selected from the group consisting of *Vibrio alginolyticus*, *V. anguillarum*, *V. campbelli*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*, *V. penaeicida*, *V. vulnificus*, *V. nereis*, *V. tubiashi*, *V. fluvialis*, *V. splendidus*, and/or *V. nigripulchritudo* or a combination of two or more thereof. In a further aspect of this method, the bacterial vaccine comprises dead and/or inactivated whole cell or cell wall fragments of PL.

[0165] In one aspect, administering comprises, consists essentially of, or consists of oral administration (e.g., feeding), injection, and/or immersion. In a further aspect, a shrimp feed is coated with the bacterial vaccine. In another aspect, the shrimp, the shrimp who produces or supports shrimp progeny, or the progeny itself are immersed in a solution or a suspension of the bacterial vaccine. In yet a further aspect, the bacterial vaccine is prepared as a solution or suspension, where an effective amount of the dead and/or inactivated bacterial vaccine composition is added to water (e.g., sterile aged brackish water or seawater), and the shrimp, the shrimp who produces or supports shrimp progeny, or the progeny itself are immersed in the solution or suspension. For example, for oral (os) vaccination, for two to seven days whether alone of following the injection vaccination. Medicated feed pellets are top-coated

with 9 mL/kg vaccine and fed at a maximum rate of 10% BW per day, dispensed over 1 meal per day.

[0166] Vaccine is mixed with potable/distilled water until homogenous. This mixture was at the intended top-coating concentration and included 20% extra volume to account for volume loss due to adhesion/cohesion and to maintain spray pressure. Homogenous solution was placed into a spray bottle and weighed. 1 kg of commercial shrimp feed was placed into a tote. By way of example Skretting SAPHIRE feed pellets (see here: <https://www.skretting.com/en-in/feed-and-services-for-aquaculture/sapphire-4853/>, last accessed on October 27, 2024) 100 g of test product solution was sprayed onto the surface of the commercial feed little by little. Feed was constantly mixed throughout.

[0167] In one aspect, the method comprises, or consists essentially of, or consists of feeding an effective amount of the bacterial vaccine composition comprising dead and/or inactivated gram-positive and/or gram-negative whole cell or cell wall fragments to the female shrimp broodstock who then passes on the immunity and/or treatment to its progeny. In a further aspect, the progeny is administered the dead and/or inactivated bacterial vaccine. In one aspect, the dead and/or inactivated bacterial vaccine formulation is contained or mixed with a typical food for the female shrimp broodstock, or the shrimp larvae, the food acting as a carrier. In one aspect, from about 1.5×10^4 to about 1.5×10^8 antigen units/gram of food is provided to or fed to the female shrimp broodstock, or the shrimp larvae.

[0168] In one aspect of this method, the administering comprises feeding, injecting, and/or submerging the female shrimp broodstock a suitable amount of bacterial vaccine, wherein the amount administered per dose of vaccine, composition or formulation comprising dead and/or inactivated gram-positive and/or gram-negative bacteria contains between about 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units, or from about 1.5×10^5 to about 1.5×10^{11} antigen units, or from about 1.5×10^6 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{10} antigen units, or from about 1.5×10^8 to about 1.5×10^9 antigen units, or at least 1.5×10^7 or 1.5×10^4 antigen units, or at least 1.5×10^5 antigen units, or at least 1.5×10^6 antigen units, or at least 1.5×10^7 antigen units, or at least about 1.5

$\times 10^8$ antigen units, or at least about 1.5×10^9 antigen units, or at least about 1.5×10^{10} antigen units, of the least one dead non-disease bacterial vaccine.

[0169] In one embodiment, the bacterial vaccine is injected into the female shrimp broodstock.

[0170] In another embodiment of this method, the administering comprises feeding a female shrimp broodstock an effective amount of the dead and/or inactivated bacterial vaccine composition, injecting the female shrimp broodstock with an effective amount of the dead and/or inactivated bacterial vaccine composition, submerging the female shrimp broodstock in an effective amount of the dead and/or inactivated bacterial vaccine composition, or any combination of two or more thereof. In an aspect, the bacterial vaccine composition comprises between about 1.5×10^4 to about 1.5×10^{11} antigen units, or from about 1.5×10^5 to about 1.5×10^{11} antigen units, or from about 1.5×10^6 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{10} antigen units, or from about 1.5×10^8 to about 1.5×10^9 antigen units, or at least 1.5×10^7 or 1.5×10^4 antigen units, or at least 1.5×10^5 antigen units, or at least 1.5×10^6 antigen units, or at least 1.5×10^7 antigen units, or at least about 1.5×10^8 antigen units, or at least about 1.5×10^9 antigen units, or at least about 1.5×10^{10} antigen units of the least one dead non-disease bacterial species.

[0171] In one embodiment of this method, the administering comprises feeding a progeny an effective amount of the dead and/or inactivated bacterial vaccine composition, injecting the progeny with an effective amount of the dead and/or inactivated bacterial vaccine composition, submerging the progeny in an effective amount of the dead and/or inactivated bacterial vaccine composition, or any combination of two or more thereof. In an aspect, the bacterial vaccine composition comprises between about 1.5×10^4 to about 1.5×10^{11} antigen units, or from about 1.5×10^5 to about 1.5×10^{11} antigen units, or from about 1.5×10^6 to about 1.5×10^{11} antigen units, or from about 1.5×10^7 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{10} antigen units, or from about 1.5×10^8 to about 1.5×10^9 antigen units, or at least 1.5×10^4 antigen units, or at least 1.5×10^5 antigen units, or at least 1.5×10^6 antigen units, or at least 1.5×10^7 antigen units, or at least about 1.5×10^8 antigen units, or at least about 1.5×10^9 antigen units, or at least about 1.5×10^{10} antigen units of the least one dead non-disease bacterial species.

10^9 antigen units, or at least about 1.5×10^{10} antigen units of the least one dead non-disease bacterial species.

[0172] In one aspect of the present invention, the vaccine composition comprises, consists essentially of, or consists of a combination vaccine prepared by mixing an effective amount of inactivated *P. larvae* and *V. parahaemolyticus* to provide protective benefits to shrimp against WSSV and/or AHPND.

[0173] *Population Treatment or Prevention in Bees and Shrimp*

[0174] *Honey Bee Population*

[0175] This disclosure provides a method for preventing or treating microbial infections and/or diseases in a population of honey bees, wherein the pathogen causing the infection is selected from the group of Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus. In one aspect, the method comprises or consists essentially of, or yet further consists of administering or feeding to the population of honey bees who produces or supports the honey bee population (colony) progeny or the honey bee progeny a bacterial vaccine formulation comprising a gram-positive bacteria. In another aspect, the method comprises, or consists essentially of, or consists of feeding an effective amount of the bacterial vaccine composition to the honey bee queen bee or honey bees that support the honey bee queen bee in the population (colony) who then passes on the immunity and/or treatment to its progeny in the population or colony. In a further aspect, the progeny in the population is administered the PL vaccine. In one aspect, the vaccine formulation is contained or mixed with a typical food for honey bees which acts as a carrier. In one aspect, from about 1.5×10^4 to about 1.5×10^8 antigen units/gram of food is provided to or fed to the honey bees in the population or colony such as the honey bee queen, worker honey bees, nurse honey bees and/or the honey bee larvae.

[0176] In one aspect of this method, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated bacterial species of the genus *Paenibacillus*. In another aspect, the dead and/or inactivated bacterial species is selected

from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericamus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvifaciens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynmii*, and/or *P. xylanilyticus* or a combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvans*, or a combination of two or more thereof. In yet another aspect, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof.

[0177] In one aspect of this method, the administering comprises feeding the honey bees in the population a suitable amount of food comprising an effective amount of dead and/or inactivated gram-positive bacteria, wherein the amount of dead and/or inactivated gram-positive bacteria administered per dose of vaccine, composition or formulation contains between about 1.5×10^4 to about 1.5×10^{11} antigen units/gram of food, or from about $1.5 \times$

10^7 or 1.5×10^5 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^6 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^7 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^{10} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^9 antigen units/gram of food, or at least 1.5×10^4 antigen units/gram of food, or at least 1.5×10^5 antigen units/gram of food, or at least 1.5×10^6 antigen units/gram of food, or at least 1.5×10^7 antigen units/gram of food, or at least about 1.5×10^8 antigen units/gram of food, or at least about 1.5×10^9 antigen units/gram of food, or at least about 1.5×10^{10} antigen units/gram of food.

[0178] In one embodiment, but not limited to the composition of oral vaccine is delivered as part of the normal husbandry practice for queen bees. The queens together with 8-10 worker bees are placed into 'queen cages' supplied with sufficient feed (queen candy) to last up to one or two weeks. The oral vaccine is added to the queen candy, on which the queen bee and the worker bees will be feeding for 3-8 days after which she is placed into the new hive, ready to lay eggs and create the new protected bee colony.

[0179] In another embodiment the vaccine is placed into the queen candy in the queen shipping box or into the nuc or queen rearing hive where nurse bees will consume the vaccine with the queen candy and transport it to their royal jelly glands where it is mixed with royal jelly that is fed to the queen bee larvae or the queen bee.

[0180] In another embodiment the vaccine is fed to the developing queen larvae during the larval rearing phase in the queen rearing hives.

[0181] This disclosure provides a method for preventing or treating a viral infection and/or disease in a population of honey bees, wherein the virus causing the viral infection is Deformed Wing Virus B (DWV-B). In one aspect, the method comprises or consists essentially of, or yet further consists of administering or feeding to the population of honey bees who produces or supports the honey bee population (colony) progeny or the honey bee progeny a dead and/or inactivated PL bacterial vaccine formulation comprises dead and inactive whole cell or cell wall fragments. In another aspect, the method comprises, or consists essentially of, or consists of feeding an effective amount of the bacterial vaccine

composition comprising dead and/or inactivated PL or fragments thereof to the honey bee queen bee or honey bees that support the honey bee queen bee in the population (colony) who then passes on the immunity and/or treatment to its progeny in the population or colony. In a further aspect, the progeny in the population is administered the dead and/or inactivated PL vaccine. In one aspect, the vaccine formulation is contained or mixed with a typical food for honey bees which acts as a carrier. In one aspect, from about 1.5×10^4 to about 1.5×10^8 antigen units/gram of food is provided to or fed to the honey bees in the population or colony such as the honey bee queen, worker honey bees, nurse honey bees and/or the honey bee larvae.

[0182] In one aspect of this method, the administering comprises feeding the honey bees in the population a suitable amount of food, wherein the amount administered per dose a vaccine, composition or formulation comprising dead and/or inactivated PL contains between about 1.5×10^4 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^7 or 1.5×10^5 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^6 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^7 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^{10} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^9 antigen units/gram of food, or at least 1.5×10^4 antigen units/gram of food, or at least 1.5×10^5 antigen units/gram of food, or at least 1.5×10^6 antigen units/gram of food, or at least 1.5×10^7 antigen units/gram of food, or at least about 1.5×10^8 antigen units/gram of food, or at least about 1.5×10^9 antigen units/gram of food, or at least about 1.5×10^{10} antigen units/gram of food, of the least one dead non-disease PL or fragments thereof.

[0183] In one embodiment, but not limited to the composition of oral vaccine is delivered as part of the normal husbandry practice for queen bees. The queens together with 8-10 worker bees are placed into 'queen cages' supplied with sufficient feed (queen candy) to last up to one or two weeks. The oral vaccine is added to the queen candy, on which the queen bee and the worker bees will be feeding for 3-8 days after which she is placed into the new hive, ready to lay eggs and create the new protected bee colony.

[0184] In another embodiment the vaccine is placed into the queen candy in the queen shipping box or into the nuc or queen rearing hive where nurse bees will consume the vaccine

with the queen candy and transport it to their royal jelly glands where it is mixed with royal jelly that is fed to the queen bee larvae or the queen bee.

[0185] In another embodiment the vaccine is fed to the developing queen larvae during the larval rearing phase in the queen rearing hives.

[0186] In a further aspect, the method further comprises testing (and such methods are known in the art) the honey bee colony or population for the virus or viral infection prior to administration of the PL vaccine, e.g., DWV-B. In a further aspect, for the treatment of virally infected population, an uninfected adult who produces progeny (uninfected queen bee) is administered the vaccine formulation and introduced into the population and vaccine formulation which is then passed on to the progeny.

[0187] *Bumble Bee Population*

[0188] This disclosure provides a method for preventing or treating a microbial infection and/or disease in a population of bumble bees. In one aspect of this method, the microbial infection is a virus selected from the group of Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus. In one aspect, the method comprises or consists essentially of, or yet further consists of administering or feeding to the population of bumble bees who produces or supports the bumble bee population (colony) progeny or the bumble bee progeny a bacterial vaccine formulation comprising dead and/or inactivated gram-positive whole cells or cell wall fragments thereof. In another aspect, the method comprises, or consists essentially of, or consists of feeding an effective amount of the bacterial vaccine composition to the bumble bee queen bee or bumble bees that support the bumble bee queen bee in the population (colony) who then passes on the immunity and/or treatment to its progeny in the population or colony. In a further aspect, the progeny in the population is administered the bacterial vaccine. In one aspect, the bacterial vaccine formulation is contained or mixed with a typical food for bumble bees which acts as a carrier. In one aspect, from about 1.5×10^4 to about 1.5×10^8 antigen units/gram of food is

provided to or fed to the bumble bees in the population or colony such as the bumble bee queen, worker bumble bees, nurse bumble bees and/or the bumble bee larvae.

[0189] In one aspect of this method, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated bacterial species of the genus *Paenibacillus*. In another aspect, the dead and/or inactivated bacterial species is selected from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericamus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvificiens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynmii*, and/or *P. xylanilyticus* or a combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvans*, or a combination of two or more thereof. In yet another aspect, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof.

[0190] In one aspect of this method, the administering comprises feeding the bumble bees in the population a suitable amount of food, wherein the amount of dead and/or inactivated gram-positive bacteria or cell wall fragments thereof administered per dose contains between about 1.5×10^4 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^7 or 1.5×10^5 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^6 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^7 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^{11} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^{10} antigen units/gram of food, or from about 1.5×10^8 to about 1.5×10^9 antigen units/gram of food, or at least 1.5×10^4 antigen units/gram of food, or at least 1.5×10^5 antigen units/gram of food, or at least 1.5×10^6 antigen units/gram of food, or at least 1.5×10^7 antigen units/gram of food, or at least about 1.5×10^8 antigen units/gram of food, or at least about 1.5×10^9 antigen units/gram of food, or at least about 1.5×10^{10} antigen units/gram of food.

[0191] In one embodiment, but not limited to the composition of oral vaccine is delivered as part of the normal husbandry practice for queen bees. The queens together with 8-10 worker bees are placed into 'queen cages' supplied with sufficient feed (queen candy) to last up to one or two weeks. The oral vaccine is added to the queen candy, on which the queen bee and the worker bees will be feeding for 3-8 days after which she is placed into the new hive, ready to lay eggs and create the new protected bee colony.

[0192] In another embodiment the vaccine is placed into the queen candy in the queen shipping box or into the nuc or queen rearing hive where nurse bees will consume the vaccine with the queen candy and transport it to their royal jelly glands where it is mixed with royal jelly that is fed to the queen bee larvae or the queen bee.

[0193] In another embodiment the vaccine is fed to the developing queen larvae during the larval rearing phase in the queen rearing hives.

[0194] In a further aspect, the method further comprises testing (and such methods are known in the art) the bumble bee colony or population for the virus or viral infection prior to administration of the PL vaccine. In a further aspect, for the treatment of infected population, an uninfected adult who produces progeny (uninfected queen bee) is administered the vaccine

and then introduced into the population and the vaccine formulation is then passed on to the progeny.

[0195] *Shrimp Population*

[0196] This disclosure provides a method for preventing or treating a microbial infection and/or disease in a population of shrimp, wherein the shrimp is selected from the group of Rock Shrimp, Pink Shrimp, Tiger Shrimp, Chinese White Shrimp, Brown Shrimp, White Shrimp, Atlantic Northern Shrimp, Spot Shrimp, Aesop Shrimp, Banana Prawn, and Blue Shrimp. In one aspect of this method, pathogen causing the infection is selected from the group of White spot syndrome virus (WSSV), Infectious hypodermal and hematopoietic necrosis virus (IHHNV), Baculovirus penaei (BP), Shrimp iridovirus (IRIDO), Taura syndrome virus (TSV), Infectious myonecrosis virus (IMNV), or Covert mortality Noda virus (CMNV) or those identified in **Tables 3 and 4**, infra; and wherein the bacterial vaccine comprises dead and/or inactivated gram-positive and/or gram-negative bacterial whole cell or cell wall fragments thereof. In a further aspect, the gram-negative bacteria is selected from *V. adaptatus*, *V. aerogenes*, *V. aestivus*, *V. aestuariamus*, *V. agarivorans*, *V. albensis*, *V. alfacensis*, *V. alginolyticus*, *V. anguillarum*, *V. areninigras*, *V. artabrorum*, *V. atlanticus*, *V. atypicus*, *V. azureus*, *V. brasiliensis*, *V. bubulus*, *V. calviensis*, *V. campbellii*, *V. casei*, *V. chagasii*, *V. cholerae*, *V. cincinnatiensis*, *V. coralliilyticus*, *V. crassostreae*, *V. cyclitrophicus*, *V. diabolicus*, *V. diazotrophicus*, *V. ezurae*, *V. fluvialis*, *V. fortis*, *V. furnissii*, *V. gallicus*, *V. gazogenes*, *V. gigantis*, *V. haliotocoli*, *V. harveyi*, *V. hepatarius*, *V. hippocampi*, *V. hispanicus*, *V. ichthyenterii*, *V. indicus*, *V. kanaloae*, *V. lentus*, *V. litoralis*, *V. logei*, *V. mediterranei*, *V. metschnikovii*, *V. mimicus*, *V. mytili*, *V. natriegens*, *V. navarrensis*, *V. neonatus*, *V. neptunius*, *V. nereis*, *V. nigripulchritudo*, *V. ordalii*, *V. orientalis*, *V. pacinii*, *V. parahaemolyticus*, *V. pectenocida*, *V. pelagius*, *V. penaeocida*, *V. pomeroyi*, *V. ponticus*, *V. proteolyticus*, *V. rotiferianus*, *V. ruber*, *V. rumoiensis*, *V. salmonocida*, *V. scophthalmi*, *V. splendidus*, *V. superstes*, *V. tapetis*, *V. tasmaniensis*, *V. tubiashii*, *V. vulnificus*, *V. wodanis*, and/or *V. xuii* or a combination of two or more thereof, or alternatively selected from the group consisting of *Vibrio alginolyticus*, *V. anguillarum*, *V. campbellii*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*, *V. penaeocida*, *V. vulnificus*, *V. nereis*, *V. tubiashi*, *V. fluvialis*, *V. splendidus*, and/or *V. nigripulchritudo* or a combination of two or more thereof. In a

further aspect, the shrimp or population thereof to be vaccinated does not include *P. polymyxa* for the vaccine or feed against *V. parahaemolyticus* infections or disease, when *P. polymyxa* is the sole active agent in the vaccine. In another aspect, the vaccine for treatment of shrimp, the colony or offspring does not include *V. anguillarum* for the treatment or prevention of infection caused by a *Vibrio* species in shrimp.

[0197] In another aspect of this method, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated bacterial species of the genus *Paenibacillus*. In another aspect, the dead and/or inactivated bacterial species is selected from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericanus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvificiens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynnii*, and/or *P. xylanilyticus* or a combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus*

xylanisolvans, or a combination of two or more thereof. In yet another aspect, the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof. In a further aspect, the bacterial vaccine comprises dead and/or inactivated bacterial species of the genus *Paenibacillus* and/or dead and/or inactivated bacterial species of the genus *Vibrio*.

[0198] In one aspect, the method comprises or consists essentially of, or yet further consists of administering to the population of female shrimp broodstock or the shrimp progeny a bacterial vaccine formulation. In one embodiment of this method, the administering comprises feeding a female shrimp broodstock or shrimp progeny an effective amount of the bacterial vaccine composition, injecting the female shrimp broodstock or the shrimp progeny with an effective amount of the bacterial vaccine composition, submerging the female shrimp broodstock or the shrimp progeny in an effective amount of the bacterial vaccine composition, or a combination of any two or more thereof.

[0199] In another aspect, the method comprises, or consists essentially of, or consists of feeding, immersing, and/or injecting an effective amount of the bacterial vaccine composition to the female shrimp broodstock who then passes on the immunity and/or treatment to its progeny in the population or colony. In a further aspect, the progeny in the population is administered the bacterial vaccine. In one aspect, the vaccine formulation is contained or mixed with a typical food for shrimp which acts as a carrier. In one aspect, from about 10^4 to about 10^8 antigen is provided to or fed to the shrimp in the population or colony such as the female shrimp broodstock, and/or the shrimp larvae.

[0200] In some embodiments, methods of administration include, but are not limited to, oral administration, injection, and immersion. In one embodiment, for use in injection administration, shrimp were injected with the vaccine composition of the invention at a dose of 10^4 - 10^8 CFU per tail. In another embodiment, for use in administration by immersion, a vaccine stock prepared by dissolving and/or suspending the vaccine composition of the present invention in water, e.g., sterile aged brackish water or seawater, and the shrimp is immersed in the solution of suspension. As an example, only the bacterial vaccine composition is added to water and is diluted in 20 L of water to achieve a concentration of 10

$2 - 10^5$ CFU / ml, soaking time is about 30 minutes or more, for example 1 hour, 2 hours, 3 hours, or more.

[0201] Accordingly, the present disclosure also provides the use of heat inactivated *Vibrio* cells as an adjuvant to improve immunity of vaccines made from formalin inactivated vibrio cells or BEI inactivated *Vibrio* cells.

[0202] In one embodiment, the composition of the vaccine is delivered via injection into the female brood stock. In yet another embodiment, the vaccine is delivered to the female brood stock and/or the shrimp progeny by feeding the female brood stock and/or the shrimp progeny feed that is coated with the vaccine composition.

[0203] *Vaccine Compositions for Treatment and Prevention of Viral and Bacterial Infections in Invertebrates*

[0204] Provided is an invertebrate vaccine and/or composition and/or formulation comprising consisting essentially of, or consisting of at least one, or at least two, or at least three, or at least four or more of dead and/or inactivated whole cell or cell wall fragments of a non-disease causing bacterial species. In one aspect, invertebrate vaccine and/or composition and/or formulation comprises, consists essentially of, or consists of at least one, or at least two, or at least three, or at least four or more of dead and/or inactivated whole cell or cell wall fragments of a non-disease causing gram-positive bacterial species and/or a gram-negative bacterial species.

[0205] Further provided is a method for preparing an invertebrate vaccine and/or composition and/or formulation comprising isolating from at least one, or at least two, or at least three, or at least four or more dead, non-disease causing bacterial species of a bacterial genus, whole cells or cell wall fragments from the least one dead, non-disease causing bacterial species as identified in **Table 1**, or alternatively **Tables 1-4** and **Experiment No. 4**.

[0206] In one embodiment, the method further comprises admixing the isolated antigen units with an insect food or carrier, a queen bee wafer or gel. In one aspect, the bacterial species is a species of the genus *Paenibacillus*, e.g., *Paenibacillus alvei* or *Paenibacillus dentritiformis*, or a combination thereof. In another aspect, the dead *Paenibacillus* species is selected from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P.*

alvei, *P. amylolyticus*, *P. anaericanus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvificiens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynmii*, and/or *P. xylanilyticus* or a combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans*-like, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvans*, or a combination thereof. In another aspect, the dead *Paenibacillus* species are *Paenibacillus alvei* and *Paenibacillus dentritiformis*.

[0207] In a further aspect, the dead and/or inactivated whole cells or cell wall fragments from the least one dead and/or inactivated, non-disease causing bacterial species selected from *V. adaptatus*, *V. aerogenes*, *V. aestivus*, *V. aestuariamus*, *V. agarivorans*, *V. albensis*, *V. alfacensis*, *V. alginolyticus*, *V. anguillarum*, *V. areninigrae*, *V. artabrorum*, *V. atlanticus*, *V. atypicus*, *V. azureus*, *V. brasiliensis*, *V. bubulus*, *V. calviensis*, *V. campbellii*, *V. casei*, *V. chagasii*, *V. cholerae*, *V. cincinattiensis*, *V. coralliilyticus*, *V. crassostreae*, *V. cyclitrophicus*, *V. diabolicus*, *V. diazotrophicus*, *V. ezurae*, *V. fluvialis*, *V. fortis*, *V. furnissii*, *V. gallicus*, *V.*

gazogenes, *V. gigantis*, *V. haliotocoli*, *V. harveyi*, *V. hepatarius*, *V. hippocampi*, *V. hispanicus*, *V. ichthyoenteri*, *V. indicus*, *V. kanaloae*, *V. lentus*, *V. litoralis*, *V. logei*, *V. mediterranei*, *V. metschnikovii*, *V. mimicus*, *V. mytili*, *V. natriegens*, *V. navarrensis*, *V. neonatus*, *V. neptunius*, *V. nereis*, *V. nigripulchritudo*, *V. ordalii*, *V. orientalis*, *V. pacinii*, *V. parahaemolyticus*, *V. pectenica*, *V. pelagius*, *V. penaeicida*, *V. pomeroyi*, *V. ponticus*, *V. proteolyticus*, *V. rotiferianus*, *V. ruber*, *V. rumoiensis*, *V. salmonicida*, *V. scopthalmi*, *V. splendidus*, *V. superstes*, *V. tapetis*, *V. tasmaniensis*, *V. tubiashii*, *V. vulnificus*, *V. wodanis*, and/or *V. xuii* or a combination of two or more thereof, or alternatively selected from the group consisting of *Vibrio alginolyticus*, *V. anguillarum*, *V. campbelli*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*, *V. penaeicida*, *V. vulnificus*, *V. nereis*, *V. tubiashi*, *V. fluvialis*, *V. splendidus*, and/or *V. nigripulchritudo* or a combination of two or more thereof. In a further aspect, the invertebrate vaccine and/or composition and/or formulation comprises dead and/or inactivated bacterial species of the genus *Paenibacillus* and/or dead and/or inactivated bacterial species of the genus *Vibrio*.

[0208] In one aspect, the whole cells or cell wall fragments of at least one, or at least two, or at least three, or at least four or more of dead, non-disease causing bacterial species of a bacterial genus per dose is provided between about 1.5×10^4 to about 1.5×10^{11} antigen units, or about 1.5×10^7 or 1.5×10^5 to about 1.5×10^{11} antigen units, or about 1.5×10^6 to about 1.5×10^{11} antigen units, or about 1.5×10^7 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{10} antigen units, or from about 1.5×10^8 to about 1.5×10^9 antigen units, or at least 1.5×10^4 antigen units, or at least 1.5×10^5 antigen units, or at least 1.5×10^6 antigen units, or at least 1.5×10^7 antigen units, or at least about 1.5×10^8 antigen units, or at least about 1.5×10^9 antigen units, or at least about 1.5×10^{10} antigen units.

[0209] Also provided is a composition comprising the dead and/or inactivated bacteria in an amount of about 1.5×10^4 to about 1.5×10^8 antigen units/gram of invertebrate food, or alternatively from between about 1.5×10^4 to about 1.5×10^{11} antigen units/gram of invertebrate food, or about 1.5×10^7 or 1.5×10^5 to about 1.5×10^{11} antigen units/gram of invertebrate food, or about 1.5×10^6 to about 1.5×10^{11} antigen units/gram of invertebrate food, or from about 1.5×10^7 to about 1.5×10^{11} antigen units/gram of invertebrate food, or

from about 1.5×10^8 to about 1.5×10^{11} antigen units/gram of invertebrate food, or from about 1.5×10^8 to about 1.5×10^{10} antigen units/gram of invertebrate food, or from about 1.5×10^8 to about 1.5×10^9 antigen units/gram of invertebrate food, or at least 1.5×10^4 antigen units/gram of invertebrate food, or at least 1.5×10^5 antigen units/gram of invertebrate food, or at least 1.5×10^6 antigen units/gram of invertebrate food, or at least 1.5×10^7 antigen units/gram of invertebrate food, or at least about 1.5×10^8 antigen units/gram of invertebrate food, or at least about 1.5×10^9 antigen units/gram of invertebrate food, or at least about 1.5×10^{10} antigen units/gram of invertebrate food.

[0210] Non-limiting examples of a carrier can be a solid or a liquid carrier and can include preservatives, insect nutrients, invertebrate nutrients, or other coloring agents as necessary. In one specific embodiment, the carrier is invertebrate food (shrimp food) or an insect food, such as a queen bee wafer or sugar feed. In an example where the carrier is an invertebrate food, the invertebrate food is coated with the bacterial vaccine and/or composition and/or formulation. In another embodiment, the carrier is suitable for injecting the bacterial vaccine and/or composition and/or formulation. In yet another embodiment, the carrier is water (e.g., sterile aged brackish water or seawater) for dissolving and/or suspending the bacterial vaccine and/or composition and/or formulation such that the invertebrate can be submerged in the solution or suspension.

[0211] In one embodiment, but not limited to the composition of oral vaccine is delivered as part of the normal husbandry practice for queen bees. The queens together with 8-10 worker bees are placed into “queen cages” supplied with sufficient feed (queen candy) to last up to one or two weeks. The oral vaccine is added to the queen candy, on which the queen bee and the worker bees will be feeding for 3-8 days after which she is placed into the new hive, ready to lay eggs and create the new protected bee colony.

[0212] In another embodiment the vaccine is placed into the queen candy in the queen shipping box or into the nuc or queen rearing hive where nurse bees will consume the vaccine with the queen candy and transport it to their royal jelly glands where it is mixed with royal jelly that is fed to the queen bee larvae or the queen bee.

[0213] In another embodiment the vaccine is fed to the developing queen larvae during the larval rearing phase in the queen rearing hives.

[0214] In some embodiments, methods of administration include, but are not limited to, oral administration, injection and/or immersion (shrimp). In one embodiment, for use in injection administration, invertebrates including bees and shrimp are injected with the vaccine composition of the invention at a dose of 10^4 - 10^8 CFU per individual. In another embodiment, for use in administration by immersion, a vaccine stock made by immersion with the vaccine composition of the present invention and administered the vaccine to shrimp, e.g., made of sterile aged brackish water or seawater in a 1 L tank can be achieved by diluting the solution in 20 L of water to achieve a concentration of 10^2 - 10^5 CFU / ml, soaking time is about 30 minutes or more, for example 1 hour, 2 hours, 3 hours, or more.

[0215] *Vaccines for Treating Vector- Borne Disease*

[0216] Vector-borne diseases (see e.g., **Table 2**) such as mosquito-borne diseases or mosquito-borne illnesses are diseases caused by bacteria, viruses, or parasites transmitted by mosquitos or other insects such as ticks. For example, nearly 700 million people contract mosquito-borne illnesses each year, resulting in more than a million deaths. Diseases transmitted by mosquitoes include malaria, dengue, West Nile virus, chikungunya, yellow fever, filariasis, tularemia, dirofilariasis, Japanese encephalitis, Saint Louis encephalitis, Western equine encephalitis, Eastern equine encephalitis, Venezuelan equine encephalitis, Ross River fever, Barmah Forest fever, La Crosse encephalitis, and Zika fever, as well as newly detected Keystone virus and Rift Valley fever.

[0217] Applicant describes herein a novel approach for immunizing and treating an insect or population thereof hosting a vector-borne disease such as a tick or Culicidae (mosquito) species including the subfamilies Culicinae and Anophelinae with an effective amount of one or more dead and/or heat-inactivated gram positive bacteria or cell wall fragments thereof. For example, Culicidae species include *Aedes albopictus*, *Aedes aegypti*, and *Aedes polynesiensis*. The dead and/or inactivated gram positive bacteria are described herein (see e.g., **Table 2**), the complete list of which is incorporated by reference herein.

[0218] In one aspect the insect is a mosquito, and the disease is selected from Chikunguna, Dengue, Rift Valley Fever, Yellow Fever, Zika, O'nyong'nyong virus, Japanese encephalitis, Zika, or West Nile Fever. In a further aspect, the disease is selected from Dengue, Rift Valley Fever, Zika, Yellow Fever, Japanese encephalitis, or West Nile Fever. In a further aspect, the insect is a mosquito, and the disease is selected from Dengue or Zika. In one aspect, the insect is a mosquito, and the disease is Zika. In a further aspect, the insect is a mosquito, and the disease is Dengue.

[0219] In another aspect, the insect is a tick, and the disease is selected from Crimean-Conglo Haemorrhagic Fever, Lyme Disease, Relapsing Fever, Rickettsial Diseases, Spotted Fever, Q Fever, Tick-borne encephalitis, or Tularaemia. In another aspect, the insect is a tick and the disease is selected from Lyme Disease, Relapsing Fever, Rickettsial Diseases, Spotted Fever, Q Fever, Tick-borne encephalitis, or Tularaemia. In another aspect, the insect is a tick, and the disease is selected from Lyme Disease, Spotted Fever, Q Fever, Tick-borne encephalitis, or Tularaemia. In another aspect, the insect is a tick, and the disease is Lyme Disease.

[0220] In one aspect, the dead and/or inactivated bacteria is a *Paenibacillus sp.*, e.g., PL. The dead and/or inactivated *Paenibacillus sp.* bacteria or cell wall fragments thereof can be microinjected into the female mosquito or mixed into humans and animal blood and fed to the insect.

[0221] Applicant's method provides for a means of generating specifically immunized and or treated insects such as tick or mosquito species as desired. The then generated immunized, treated insects such as tick or mosquitoes can be employed to control the infection pressure in a insects such as tick or mosquito population by introducing a specific species immunized, treated with an effective amount of the dead and/or inactivated bacteria or cell wall fragments thereof into a population of insects such as tick or mosquitoes to suppress the ability of the population to become effective diseases vectors and reduce the risk of transmission on an infection to another species such as animals, humans or plants.

[0222] Applicant also provides a method of immunizing or treating insects such as tick, e.g., *Aedes* mosquito species with a dead bacterium comprising injecting or feeding a female insect such as a tick or mosquito, e.g., an *Aedes* mosquito with dead and/or inactivated

bacteria or cell wall fragments thereof a *Paenibacillus sp.* In one aspect the dead or inactivated bacteria is PL, or cell wall fragments thereof. The *Aedes* mosquito species includes an *Aedes* mosquito species selected from the group comprising of *Aedes albopictus*, *Aedes aegypti* and *Aedes polynesiensis*.

[0223] Also provided herein is a method of lowering the ability to for the treated and immunized animal to get infectious disease including bacterial and viral diseases such as Zika, Dengue (see **Tables 1 and 2**) and to become a vector for such bacteria and viruses by administering to the animal a dead and/or inactivated bacteria or cell wall fragments thereof as described herein, e.g., of a *Paenibacillus sp.* In one aspect the dead or inactivated bacteria is PL, or cell wall fragments thereof. The methods of this disclosure can also prevent infection in the next generation by immunizing or treating the maternal insect, e.g., a tick or mosquito.

[0224] The present method uses dead and/or inactivated gram positive bacteria, e.g., of *Paenibacillus sp.* or cell wall fragments thereof for the suppression/elimination of disease causing viral and bacterial levels in insects harboring vector-borne diseases, e.g., ticks or mosquitoes and strategies that reduce or block pathogen transmission by insects harboring vector-borne diseases, e.g., ticks or mosquitoes as a means to infection disease control. These strategies include vector suppression and replacement based upon immunization, treatment of maternal insects harboring vector-borne diseases, e.g., maternal ticks or maternal mosquitos with dead and/or inactivated *Paenibacillus sp.* bacteria or cell wall fragments thereof. One mechanism is transgenerational immune priming through which immunization / treatment of the maternal insect passes the immunity to infections to the next generation and thereby lowers or prevents the ability to get infected by viruses and bacteria and thereby lowering the ability to become a disease vector and spread of infectious disease by the female's offspring.

[0225] It will be apparent to one of ordinary skill in the art that the present method can be used to protect a population of insects harboring vector-borne diseases, e.g., ticks or mosquitoes including F1 offspring from harboring effective levels of infectious disease agents including virus and bacteria by using the present method to treat, immunize female mosquitoes with one or more dead and/or inactivated *Paenibacillus sp.* bacteria or cell wall fragments thereof strains and introducing those immunized and treated female insects

harboring vector-borne diseases, e.g., ticks or mosquitos into a population. In one embodiment, the dead and/or inactivated *Paenibacillus sp.* bacteria or cell wall fragments thereof is PL. As the female insect harboring vector-borne diseases, e.g., tick or mosquito mates and releases eggs the resulting insects harboring vector-borne diseases, e.g., ticks or mosquitos will be effectively protected from becoming infected when consuming blood that contains infectious disease agents.

[0226] The present method aids in controlling the growing burden of vector-borne disease by effectively immunizing, treating a population in which a natural vector population cannot become infected and a carrier of disease.

[0227] Provided is an invertebrate vaccine and/or composition and/or formulation comprising consisting essentially of, or consisting of at least one, or at least two, or at least three, or at least four or more of dead and/or inactivated whole cell or cell wall fragments of a non-disease causing bacterial species. In one aspect, invertebrate vaccine and/or composition and/or formulation comprises, consists essentially of, or consists of at least one, or at least two, or at least three, or at least four or more of dead and/or inactivated whole cell or cell wall fragments of a non-disease causing gram-positive bacterial species and/or a gram-negative bacterial species. In one aspect the composition or formulation comprises *Paenibacillus sp.* bacteria or cell wall fragments thereof, e.g. as described above. In a further aspect the *Paenibacillus sp.* bacteria or cell wall fragments thereof is PL or cell wall fragments thereof.

[0228] Further provided is a method for preparing an invertebrate vaccine and/or composition and/or formulation comprising isolating from at least one, or at least two, or at least three, or at least four or more dead, non-disease causing bacterial species of a bacterial genus, whole cells or cell wall fragments from the least one dead, non-disease causing bacterial species as identified in **Table 1**.

[0229] In one aspect, the bacterial species is a species of the genus *Paenibacillus*, e.g., *Paenibacillus alvei* or *Paenibacillus dentritiformis*, or a combination thereof. In another aspect, the dead *Paenibacillus* species is selected from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P.*

anaericanus, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvifaciens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynnii*, and/or *P. xylanilyticus* or a combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans*-like, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvans*, or a combination thereof. In another aspect, the dead *Paenibacillus* species are *Paenibacillus alvei* and *Paenibacillus dentritiformis*. In another aspect, the bacteria is PL.

[0230] In one aspect, the whole cells or cell wall fragments of at least one, or at least two, or at least three, or at least four or more of dead, non-disease causing bacterial species of a bacterial genus per dose is provided between about 1.5×10^4 to about 1.5×10^{11} antigen units, or about 1.5×10^7 or 1.5×10^5 to about 1.5×10^{11} antigen units, or about 1.5×10^6 to about 1.5×10^{11} antigen units, or about 1.5×10^7 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{11} antigen units, or from about 1.5×10^8 to about 1.5×10^{10} antigen units, or from about 1.5×10^8 to about 1.5×10^9 antigen units, or at least 1.5×10^4 antigen units, or at least 1.5×10^5 antigen units, or at least 1.5×10^6 antigen units, or at least 1.5×10^7

antigen units, or at least about 1.5×10^8 antigen units, or at least about 1.5×10^9 antigen units, or at least about 1.5×10^{10} antigen units.

[0231] In another aspect, the dead and/or inactivated bacteria is administered in an amount of about 1.5×10^4 to about 1.5×10^8 antigen units/gram of invertebrate food, or alternatively from between about 1.5×10^4 to about 1.5×10^{11} antigen units/gram of invertebrate food, or about 1.5×10^7 or 1.5×10^5 to about 1.5×10^{11} antigen units/gram of invertebrate food, or about 1.5×10^6 to about 1.5×10^{11} antigen units/gram of invertebrate food, or from about 1.5×10^7 to about 1.5×10^{11} antigen units/gram of invertebrate food, or from about 1.5×10^8 to about 1.5×10^{11} antigen units/gram of invertebrate food, or from about 1.5×10^8 to about 1.5×10^{10} antigen units/gram of invertebrate food, or from about 1.5×10^8 to about 1.5×10^9 antigen units/gram of invertebrate food, or at least 1.5×10^4 antigen units/gram of invertebrate food, or at least 1.5×10^5 antigen units/gram of invertebrate food, or at least 1.5×10^6 antigen units/gram of invertebrate food, or at least 1.5×10^7 antigen units/gram of invertebrate food, or at least about 1.5×10^8 antigen units/gram of invertebrate food, or at least about 1.5×10^9 antigen units/gram of invertebrate food, or at least about 1.5×10^{10} antigen units/gram of invertebrate food.

[0232] Non-limiting examples of a carrier can be a solid or a liquid carrier and can include preservatives, insect nutrients, invertebrate nutrients, or other coloring agents as necessary. In another embodiment, the carrier is suitable for injecting the bacterial vaccine and/or composition and/or formulation. In yet another embodiment, the carrier is water (e.g., sterile aged brackish water or seawater) for dissolving and/or suspending the bacterial vaccine and/or composition and/or formulation such that the invertebrate can be submerged in the solution or suspension.

[0233] Experiment No. 1

[0234] *Vaccination Preparation and Inoculation of Bees*

[0235] The vaccine preparation was derived from a *P. larvae* strain, originally isolated in 2018 from a New York honeybee colony. The vaccine was a proprietary aqueous suspension of inactivated *P. larvae* vegetative stage bacilli provided by Diamond Animal Health. The bacterin has passed all regulatory testing of purity and was tested and found to be of ERIC I

genotype. Bacteria were enumerated by flow cytometry and OD600 before inactivation. The bacterin was blended with queen feed (48 ml corn syrup per 500 g powdered sugar) at a ratio of 1 ml per 100 g (or control using 1 ml of water per 100 g queen feed).

[0236] *Innoculation*

[0237] A field experiment using 200 vaccinated and 200 unvaccinated colonies was conducted. Applicant showed that immediately prior to vaccination, adult honey bees from control and experimental colonies have statistically identical quantities of DWV-B. 4 months post vaccination, it was shown that adult bees from experimental colonies have significantly reduced quantities of DWV-B compared to control colonies.

[0238] Young adult (nurse) bees from 8 study sites were used at both timepoints. At each site nurse honey bees from 10 control colonies and 10 vaccinated colonies were pooled. Nurse honey bees were then sent to the National Agricultural Genotyping Center where presence of DWV-B was quantified using PCR. There were lower levels of DWV-B in vaccinated colonies compared to control colonies in all 8 sites after, but not before, vaccination. On average, DWV-B presences was reduced by 83% in each yard, with a high of 99.86%, and a low of 20% reduction.

[0239] By way of example only, Applicant has shown significantly decreased DWV-B viral load in samples of honey bees pooled from colonies with honey bee queens that have been vaccinated with *Paenibacillus larvae* bacterin compared to colonies with unvaccinated queens. Treatment groups had identical DWV-B loads prior to vaccination, and differences were apparent at 4 months post vaccination.

[0240] Experiment No. 2

[0241] *Reduction of Deformed Wing Virus - B levels in Honey Bee Colonies after Queen Vaccination with inactivated Paenibacillus larvae*

[0242] Deformed Wing Virus (DWV), an RNA virus in the family Iflaviridae, is one of the most widespread and destructive diseases affecting honey bees (Chen & Siede, 2007; de Miranda & Genersch, 2010), with studies commonly finding DWV in over 90% of tested colonies (Natsopoulou et al., 2017; Kevill et al., 2019; Paxton et al., 2022). Pupae infected

with DWV develop into abnormal adult bees with undeveloped non-functional wings, bloated abdomens, decreased adult size, and severely reduced lifespan (de Miranda & Genersch, 2010). There is evidence that even subclinical levels of DWV in bees can impair cognitive function, reduce foraging efficacy, and reduce lifespan (Benaets et al., 2017; Chen et al., 2021).

[0243] DWV is split into 3 main strains: the common DWV-A (Lanzi et al., 2006) and DWV-B (Ongus et al., 2004), and the less common DWV-C (Mordecai et al., 2016). While DWV-A has historically been most prevalent, DWV-B is rapidly displacing it worldwide in association with the global spread of *Varroa destructor* mites. (Paxton et al., 2022). DWV-B accumulates at higher levels in bees and brood (Norton et al., 2020) potentially because it is more easily transmitted by *Varroa destructor* than DWV-A (Ryabov et al., 2014, 2019). As a result of their extensive pathology, both DWV-A and DWV-B have been directly linked to overwintering failure (Kevill et al., 2019; Natsopoulou et al., 2017), and are of tremendous concern to beekeepers.

[0244] DWV is highly associated with the obligate honey bee ectoparasite mite, *Varroa destructor* (Wilfert et al., 2016; Barroso-Arévalo et al., 2019; Posada-Florez et al., 2019) the factor beekeepers most strongly associate with colony loss (Engebretson et al., 2022). *Varroa* mites parasitically feed on the fat body tissue of both developing pupae and adult bees (Ramsey et al., 2019; Warner et al., 2024) making them an effective vector for DWV transmission (Natsopoulou et al., 2017; Posada-Florez et al., 2019). *Varroa* mites also increase the severity of DWV infection by weakening their host's immune system through down regulation of host immune gene expression (Nazzi et al., 2012). In the absence of *Varroa*, DWV persists in colonies through vertical transmission from infected queens to their offspring, or by direct horizontal transmission via trophallaxis or larval feeding (Yue & Genersch, 2005; Yue et al., 2007). Typically, miteless transmission produces low viral loads, and results in subclinical DWV (Locke et al., 2017).

[0245] Studies have shown that activating the RNAi-system by feeding virus-specific dsRNA to larvae or adult bees before infection with DWV or Israeli Acute Paralysis Virus (IAPV) reduces the viral load, mortality, and symptoms resulting from the specific viral infection (Hunter et al., 2010; Desai et al., 2012). Potassium ion channel manipulation has also been

proven effective treatment for IAPV (Fellows et al., 2023). Neither of these methods, however, are currently approved for use in the field, and there is no licensed specific treatment or prophylaxis for DWV (Smeele et al., 2023). Currently, the primary method of reducing DWV levels in a colony is vector control through the reduction of Varroa levels (Woodford et al., 2022). This is generally done using insecticides and acaricides like amitraz (Warner et al., 2024) which are widely used in agriculture for pest control. However, when used in honey bees, these chemicals can reduce viral immunity (O'Neal et al., 2017) and contaminate honey (Pohorecka et al., 2018).

[0246] An alternate mechanism for direct disease management in honey bee colonies is protection through vaccination. Currently, vaccination using the *Paenibacillus larvae* bacterin is commercially approved for protection of honey bee larvae against American Foulbrood, through Trans Generational Immune Priming (TGIP), the parent-to-offspring transfer of immune experience (Hernández López et al., 2014; Dickel et al., 2022). Vaccinating parent insects with killed pathogens has been shown to provide homologous protection through TGIP across a wide range of invertebrate lineages (Tetreau et al., 2019) including honey bees (Freitak et al., 2014; Hernández López et al., 2014). It has been hypothesized that vaccination using inactivated DWV- A could provide trans-generational protection, though results to date are mixed, with context- dependent TGIP benefits recorded after venereal transmission of DWV-A to queen bees (Lang et al., 2022), but a lack of benefits after oral administration (Leponiemi et al., 2021).

[0247] Here, Applicant presents the first evidence that oral vaccination of a queen bee with an inactivated gram-positive bacteria, *Paenibacillus larvae* can directly reduce DWV-B levels in honey bee colonies in the field, independent of Varroa destructor burden. These data are to our knowledge the first use of TGIP with a killed bacteria to provide heterologous protection against a virus, and the first example of control of DWV in the field independent of mite control.

[0248] *Methods*

[0249] *Vaccination of Queens*

[0250] On May 31, 2023, 400 Italian honey bee queens, *Apis mellifera ligustica*, were sourced from Vidalia Apicultural Services in Toombs County GA. 200 queens were vaccinated with fully inactivated *P. larvae bacterin* (PCN 2915.00, produced according to the proprietary outline of production of Diamond Animal Health) and 200 remained unvaccinated. For every 50 bees to be vaccinated, 3mL of *P. larvae bacterin* solution was added to 300g of queen candy (approx. 1:7.5 w/w corn syrup to powdered sugar), resulting in 1.5×10^8 dead *P. larvae* cells per queen. Queens were isolated in queen cages and placed battery boxes in groups of 50. 300g of candy containing the bacterin was laid out in two strips between the queen cages in each battery box. Approximately 2100 nurse bees were then placed in the battery box to attend to the queens. Queens and nurses were left in the battery boxes for 8 days to allow for total consumption of the candy, before being placed into hives. Due to difficulties in procuring 400 queens on the same day, the 200 unvaccinated queens did not go through the vaccination protocol and are therefore true controls and not placebo controls. Requeening of all hives occurred on June 8, 2023.

[0251] *Subjects*

[0252] In May 2023, 400 established honey bee colonies across 8 yards in Toombs County Georgia were entered into the study. Yards contained between 39 and 78 colonies, which were divided roughly evenly into vaccinated and unvaccinated. Prior to study onset, colonies were inspected to verify they were robust and free of pests and disease before entry into the trial. At the start of the study, each colony consisted of one deep and one medium hive body. Most colonies maintained this size through the study, however some expansions and contractions were performed by yard crews in line with their standard hive management practices. Colonies were treated for varroa mites monthly using amitraz, and were fed sugar water during periods of low nectar flow to supplement their nectar intake.

[0253] *Sample Collection*

[0254] 10 nurse bees were collected from 100 vaccinated and 100 unvaccinated colonies immediately before experimental queens were placed into colonies in May 2023, and then again 4 months after queens were placed in colonies in September 2023. Nurse bees were collected from above the brood comb, and were visually identified by their coloration and

intact dorsal hairs. Nurse bees were placed into 15ml falcon tubes and flash frozen on dry ice in the field. Bees were then stored in a -80C freezer in the lab until submission.

[0255] *Sample Analysis*

[0256] In all 8 yards, a single pooled sample of nurse bees was sent for analysis from each experimental group. Each pooled sample contained 50 bees collected equally from 10 colonies (N=8 samples/ treatment/ timepoint).

[0257] Blinded samples were sent to and analyzed by the National Agricultural Genotyping Center (NAGC) in Fargo SD, an accredited testing facility used by beekeepers and scientists across North America. DWV-B levels were analyzed using qPCR. Samples were tested against a positive control, reagent control, and a no template control. PCR cycling conditions are proprietary to the NAGC and therefore cannot be reported here.

[0258] Samples were tested using similar methods for DWV-A, DWV-C, American Foulbrood, European Foulbrood, and Sacbrood Virus, but little to no instances of these disease were observed, and those results are therefore not reported.

[0259] *Mite Quantification*

[0260] Mites were collected from colonies both in May and November 2023. Counts were taken using a Veto-pharma Varroa Easy Check kit. Approximately 300 bees were collected from above the brood comb in sampled hives and washed in 70% ethanol to separate the mites from the bees. The quantity of mites beneath the sieve were quantified and are reported as the number of mites/100 bees. Washes were taken from colonies In May (N=35 unvaccinated, N=38 vaccinated) and in November (N=44 vaccinated and N=44 unvaccinated).

[0261] *Statistical Analysis*

[0262] DWV-B levels were compared using a Wilcox rank sum test because they were exponentially distributed. Mite counts were compared using a welch two sample T-test. All statistical analyses were conducted in R version 4.3.0 (R Core Team, 2024).

[0263] **Results**

[0264] *DWV-B levels*

[0265] DWV-B levels were identical between groups one week prior to vaccination (Wilcoxon rank sum test, $W=31$, $P=0.96$), and were significantly reduced in vaccinated hives compared to control hives 4 months post vaccination (Wilcoxon rank sum test, $W=54.5$, $P=0.021$) (Figure 1). DWV-B quantities were reduced in vaccinated colonies compared to unvaccinated colonies in all 8 yards, with an average reduction of 83%.

[0266] *Mite Counts*

[0267] There was no difference in mite counts between treatments one week prior to vaccination (Welch two sample t-test, $T=0.38$, $df=61$ $P=0.71$) or six months post vaccination (Welch two sample t-test, $T=-1.30$, $df=48$ $P=0.20$) (Figure 2). Following standard beekeeping practice, all colonies were regularly treated for mites using amitraz. These results indicate there was no difference in mite load between treatment groups.

[0268] **Discussion**

[0269] This study provides the first evidence that vaccinating honey bee queens *with Penibacillus larvae* bacterin provides heterologous trans-generational protection against DWV-B in a field setting. These data are also to our knowledge the first evidence that vaccinating an organism with killed bacteria can provide protection against a viral disease in an organism possessing only an innate immune system. The reduction in viral load occurred despite there being no difference in *Varroa destructor* quantities between vaccinated and unvaccinated colonies, suggesting the effect is independent of mite load. This experiment was conducted on full size honey bee colonies at a commercial apiary, and involved no special colony management practices outside of providing colonies with vaccinated queens. These results indicate that vaccinating queens with the *P. larvae* bacterin may be an efficient method to directly control DWV-B in honey bee colonies in the field.

[0270] Most laboratory studies on DWV are undertaken in the absence of *Varroa destructor*, which are present in honey bee colonies worldwide, nearly impossible to eradicate, highly associated with increased DWV levels, and directly weaken and disrupt bees' immune response (Annoscia et al., 2019; Kuster et al., 2014; Ramsey et al., 2019). Previous studies investigating homologous trans-generational protection against DWV in the lab have either failed to show protection (Leponiemi et al., 2021), or showed protection only under specific

circumstances (Lang et al., 2022). That Applicant observed heterologous protection against DWV-B after vaccinating queen bees with the *P. larvae bacterin* was therefore surprising. Arthropods, including honey bees, do not possess an adaptive immune system (Baxter et al., 2017) and rely solely on innate immunity for protection against pathogens (Morfin et al., 2021). It has been noted that honey bees express only 1/3 as many immune-regulating genes as solitary insects, and instead rely more on social immunity such as grooming, task separation and thermoregulation. This reduction in genes may increase “cross-talk” between immunologic pathways, and perhaps results in less specific immunologic responses which would increase the likelihood vaccination causes a generalized immune benefit.

[0271] While Applicant have not yet determined the mechanism by which bacterial inoculation helps reduce viral infection, Applicant speculate that the vaccine may be augmenting one or more of the common pathways of the innate bee immune system, thereby causing an increase in innate immune function of bee larvae (Nazzi & Le Conte, 2016; Annoscia et al., 2019). Innate immune responses often function by increasing expression of generalized defenses like AMPs and siRNAs (Kingsolver et al., 2013; Mondotte et al., 2020). It has been shown that bumble bee queens injected with heat-killed bacteria pass trans-generational immune benefits to their daughters, who upregulate genes associated with AMPs and toll signaling pathways, even in the absence of infection (Barribeau et al., 2016). Simply altering immune function, however, does not guarantee a decrease in disease. An upregulation of both RNAi genes and siRNA activity are observed in response to DWV infection, which does not inhibit DWV from accumulating to high levels (Norton et al., 2023). This may be in part due to an immunosuppression syndrome associated with DWV infection, which includes strong down-regulation of NF- κ B, a transcription factor which helps protect against a wide range of environmental challenges (Nazzi et al., 2012). Future transcriptomic studies should look for modifications honey bee worker gene regulation after queens are inoculated with the *P. larvae bacterin* to shed light on how these complex interactions may be affecting bee immunity.

[0272] If the *P. larvae bacterin* does indeed improve general immune function, Applicant would expect to see similar heterologous protection against a wide range of disease in the

field. While Applicant tested for protection against 5 other diseases, 3 were not detected, and 2 were only present at subclinical levels where efficacy could not be assessed.

[0273] Experiment No. 3

[0274] Vaccines for Crustaceans and Shrimp

[0275] Vaccine Preparation

[0276] A vaccine treatment prepared by heat inactivated *P. larvae* and *V. parahaemolyticus* is used to vaccinate groups of broodstock *L. vannamei* to assess whether a bacterin prepared from a major shrimp pathogen, *V. parahaemolyticus* and *P. larvae* can provide non-specific protection against WSSV and AHPND challenges of progeny shrimp. The vaccine strain of *P. larvae* is a Gram-positive pathogen of honeybee. Whereas *V. parahaemolyticus* is a known Gram-negative pathogen of shrimp, and certain strains of this bacteria are involved in the pathogenicity of AHPND in shrimp.

[0277] Heat-inactivated vaccines from *P. larvae* and *V. parahaemolyticus* were prepared. *P. larvae* were grown in MYPGP (Mueller-Hinton broth, yeast extract, potassium phosphate, glucose and pyruvate) broth at 37°C ± 2°C incubator at 250 ± 50 rpm for three (3) days and are inactivated by autoclaving at 121°C for 15 minutes. The inactivation was confirmed by plating on MYPGP agar plates (10 plates) and incubating at 37°C for 14 days and were observed for any growth of visible colonies. Additionally, plating on TSA blood agar plates (2 plates) and incubating at 37°C for 24-48 hours was done and observed for any growth of visible colonies. No growth on all inoculated plates confirmed sterility.

[0278] Similarly, *V. parahaemolyticus* was grown in Marine 2216 broth at 37°C ± 2°C incubator at 250 ± 50rpm for one (1) day. The exponentially growing culture was inactivated by autoclaving at 121°C for 15 minutes. The inactivation was confirmed by plating on Marine 2216 agar plates (10 plates) and incubating at 37°C for 14 days and were observed for any growth of visible colonies. Additionally, plating on TSA blood agar plates (2 plates) and incubating at 37°C for 24-48 hours was done and observed for any growth of visible colonies. No growth on all inoculated plates confirmed sterility. Heat inactivated vaccines were stored at 2-8°C until used for vaccination.

[0279] Feed Premixing with Vaccines or Placebo

[0280] Commercial shrimp pellet feeds were coated with either the vaccine treatments or placebo. The vaccine treatments at a dose of $>10^9$ cells/mL were coated to saturate the feed pellet. Placebo (sterile culture medium) treatment was coated onto pellet feeds similarly. Premixed feed treatments were stored at 2-8°C until used for vaccination.

[0281] *Broodstock shrimp*

[0282] Selected specific pathogen free (SPF) brood stock white shrimps (*Litopenaeus vannamei*) were vaccinated by different vaccine preparations as shown in **Table 5**. Healthy female brood stock shrimp were selected, tagged by eye tagging and checked regularly to determine ovary development stage. The SPF status was checked for major shrimp pathogens by standard PCR methods. Enrolled shrimp were weighed counted and allocated to 90 L broodstock tanks (4-6 females in duplicate tanks/treatment). All major water quality parameters were checked regularly, and routine husbandry practices were followed as per standard procedures at the testing facility. Shrimp were fed a combination of live brood stock feeds and pellet feed.

[0283] **Table 5. Experimental design.**

No.	Treatment Group	No. of reps.	Test Materials	AHPND Challenge	EMS Challenge
1	Negative control*	3		No	No
2	Positive control**	3		Yes	Yes
3	G1	3	Product PL-i.m	Yes	Yes
4	G2	3	Product PL-i.m/per os	Yes	Yes
5	G3	3	Product PL/PV-i.m	Yes	Yes
6	G4	3	Product PL/PV-i.m/per os	Yes	Yes

7	G5	3	Placebo -i.m	Yes	Yes
8	G6	3	Placebo -i.m/per os	Yes	Yes

[0284] *Vaccination or Placebo treatment*

[0285] Just a few days before the females were ready to spawn (determined based on eye observation of ovarian development), on Day 4, the shrimp were injected (intramuscular injection to the dorsal musculature) with respective treatments (**Table 5**).

[0286] In addition certain groups were also fed by the vaccine premixed feed treatments or placebo feed consecutively for four days as shown in Table 5 (on Day 0, 1, 2, 3 of the trial).

[0287] Shrimps in each treatment tank were allowed to mate with male shrimps in appropriate proportions following standard shrimp husbandry procedures. Offspring larvae produced from each treatment group were reared separately, fed following standard husbandry practices and followed up until they reach appropriate size for challenging with the challenge organisms.

[0288] *Challenge with AHPND and WSSV*

[0289] *Challenge in 1-2 g sized shrimp.*

[0290] When the offspring shrimp reached 1-2 g size, appropriate number of juvenile shrimps from each treatment group were divided into two challenge groups and challenged with either AHPND or WSSV separately (Table 5). Clinical signs of disease and daily mortality in each challenge group were observed for 12 days for AHPND and 16 days for WSSV days.

[0291] *Second challenge in 10 g sized shrimp*

[0292] The remaining unchallenged offspring shrimp from each treatment group were allowed to grow until they reached approximately 10 g size. Then appropriate number of shrimps from each treatment group were divided into two challenge groups and challenged again as described above with AHPND and WSSV.

[0293] *Determination of the cause of mortality in AHPND challenge groups*

[0294] Mortalities were monitored on a daily basis for 12 days and re-isolation of AHPND causing challenged *V. parahaemolyticus* strain were performed by sampling 20% of the daily mortality and aseptically streaking hepatopaneas samples on Marine 2216E agar and incubating plates at 347 °C for 24 hours. Presumptive *V. parahaemolyticus* challenge strains reisolated were confirmed by AHPND specific PCR testing.

[0295] *Determination of the cause of mortality in WSSV challenge groups*

[0296] Mortalities were monitored on a daily basis for 16 days and re-isolation of AHPND causing challenged WSSV strain was attempted by sampling 20% of the daily mortality and testing hepatopaneas samples using WSSV specific PCR method.

[0297] *Discussion*

[0298] Cumulative percent mortality (CPM) of each vaccine treatment or placebo group during the days after challenge was calculated. The relative percent survival (RPS) was calculated for the vaccinated groups using the following formula:

[0299] $(1 - [\text{CPM in vaccinated group} / \text{CPM in unvaccinated placebo group}]) \times 100$

[0300] Differences in CPM was determined using a one-way ANOVA ($\alpha = 0.05$) after confirming residuals are normally distributed and variances are equal for data sets. If the differences are significant ($P < 0.05$), a Tukey's post-hoc test was carried out to determine which groups are different. Statistical analysis was done using GraphPad®Prism (or other appropriate software).

[0301] **Experiment No. 4**

[0302] *Vaccines for Treating Vector-Borne Diseases Such as Mosquito-Borne or Tick-Borne Disease*

[0303] The present method aids in controlling the growing burden of vector-borne disease by effectively immunizing, treating a population in which a natural vector population cannot become infected and a carrier of disease.

[0304] In one aspect the insect is a mosquito and the disease is selected from Chikunguna, Dengue, Rift Valley Fever, Yellow Fever, Zika, O'nyong'nyong virus, Japanese encephalitis, Zika, or West Nile Fever. In a further aspect, the disease is selected from Dengue, Rift Valley Fever, Zika, Yellow Fever, Japanese encephalitis, or West Nile Fever. In a further aspect, the insect is a mosquito, and the disease is selected from Dengue or Zika. In one aspect, the insect is a mosquito, and the disease is Zika. In a further aspect, the insect is a mosquito, and the disease is Dengue.

[0305] In one aspect, the bacterial species that is administered is a species of the genus *Paenibacillus*, e.g., *Paenibacillus alvei* or *Paenibacillus dentritiformis*, or a combination thereof. In another aspect, the dead *Paenibacillus* species is selected from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericanus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdianolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvifaciens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynnii*, and/or *P. xylanilyticus* or a combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans*-like, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*,

Paenibacillus stellife, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvans*, or a combination thereof. In another aspect, the dead *Paenibacillus* species are *Paenibacillus alvei* and *Paenibacillus dentritiformis*.

[0306] In a further aspect, the dead and/or inactivated whole cells or cell wall fragments from the least one dead and/or inactivated, non-disease causing bacterial species selected from *V. adaptatus*, *V. aerogenes*, *V. aestivus*, *V. aestuariamus*, *V. agarivorans*, *V. albensis*, *V. alfacensis*, *V. alginolyticus*, *V. anguillarum*, *V. areninigras*, *V. artabrorum*, *V. atlanticus*, *V. atypicus*, *V. azureus*, *V. brasiliensis*, *V. bubulus*, *V. calviensis*, *V. campbellii*, *V. casei*, *V. chagasii*, *V. cholerae*, *V. cincinnatiensis*, *V. coralliilyticus*, *V. crassostreae*, *V. cyclitrophicus*, *V. diabolicus*, *V. diazotrophicus*, *V. ezurae*, *V. fluvialis*, *V. fortis*, *V. furnissii*, *V. gallicus*, *V. gazogenes*, *V. gigantis*, *V. haliotocoli*, *V. harveyi*, *V. hepatarius*, *V. hippocampi*, *V. hispanicus*, *V. ichthyenterii*, *V. indicus*, *V. kanaloae*, *V. lentus*, *V. litoralis*, *V. logei*, *V. mediterranei*, *V. metschnikovii*, *V. mimicus*, *V. mytili*, *V. natriegens*, *V. navarrensis*, *V. neonatus*, *V. neptunius*, *V. nereis*, *V. nigripulchritudo*, *V. ordalii*, *V. orientalis*, *V. pacinii*, *V. parahaemolyticus*, *V. pectenica*, *V. pelagius*, *V. penaeicida*, *V. pomeroyi*, *V. ponticus*, *V. proteolyticus*, *V. rotiferianus*, *V. ruber*, *V. rumoiensis*, *V. salmonicida*, *V. scophthalmi*, *V. splendidus*, *V. superstes*, *V. tapetis*, *V. tasmaniensis*, *V. tubiashii*, *V. vulnificus*, *V. wodanis*, and/or *V. xuii* or a combination of two or more thereof, or alternatively selected from the group consisting of *Vibrio alginolyticus*, *V. anguillarum*, *V. campbelli*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*, *V. penaeicida*, *V. vulnificus*, *V. nereis*, *V. tubiashi*, *V. fluvialis*, *V. splendidus*, and/or *V. nigripulchritudo* or a combination of two or more thereof. In a further aspect, the invertebrate vaccine and/or composition and/or formulation comprises dead and/or inactivated bacterial species of the genus *Paenibacillus* and/or dead and/or inactivated bacterial species of the genus *Vibrio*.

[0307] In another aspect, the insect is a tick and the disease is selected from Crimean-Congole Hemorrhagic Fever, Lyme Disease, Relapsing Fever, Rickettsial Diseases, Spotted Fever, Q Fever, Tick-borne encephalitis, or Tularaemia. In another aspect, the insect is a tick and the disease is selected from Lyme Disease, Relapsing Fever, Rickettsial Diseases, Spotted Fever, Q Fever, Tick-borne encephalitis, or Tularaemia. In another aspect, the insect is a tick and the

disease is selected from Lyme Disease, Spotted Fever, Q Fever, Tick-borne encephalitis, or Tularaemia. In another aspect, the insect is a tick and the disease is Lyme Disease.

[0308] In one aspect, the bacterial species that is administered is a species of the genus *Paenibacillus*, e.g., *Paenibacillus alvei* or *Paenibacillus dentritiformis*, or a combination thereof. In another aspect, the dead *Paenibacillus* species is selected from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvificiens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynnii*, and/or *P. xylanilyticus* or a combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans*-like, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvans*, or a combination thereof. In another aspect, the dead *Paenibacillus* species are *Paenibacillus alvei* and *Paenibacillus dentritiformis*.

[0309] In a further aspect, the dead and/or inactivated whole cells or cell wall fragments from the least one dead and/or inactivated, non-disease causing bacterial species selected from *V.*

adaptatus, *V. aerogenes*, *V. aestivus*, *V. aestuarianus*, *V. agarivorans*, *V. albensis*, *V. alfacensis*, *V. alginolyticus*, *V. anguillarum*, *V. areninigræ*, *V. artabrorum*, *V. atlanticus*, *V. atypicus*, *V. azureus*, *V. brasiliensis*, *V. bubulus*, *V. calviensis*, *V. campbellii*, *V. casei*, *V. chagasii*, *V. cholerae*, *V. cincinnatiensis*, *V. coralliilyticus*, *V. crassostreae*, *V. cyclitrophicus*, *V. diabolicus*, *V. diazotrophicus*, *V. ezurae*, *V. fluvialis*, *V. fortis*, *V. furnissii*, *V. gallicus*, *V. gazogenes*, *V. gigantis*, *V. haliotocoli*, *V. harveyi*, *V. hepaticus*, *V. hippocampi*, *V. hispanicus*, *V. ichthyenteri*, *V. indicus*, *V. kanaloae*, *V. lentus*, *V. litoralis*, *V. logei*, *V. mediterranei*, *V. metschnikovii*, *V. mimicus*, *V. mytili*, *V. natriegens*, *V. navarrensis*, *V. neonatus*, *V. neptunius*, *V. nereis*, *V. nigripulchritudo*, *V. ordalii*, *V. orientalis*, *V. pacinii*, *V. parahaemolyticus*, *V. pectenica*, *V. pelagius*, *V. penaeicida*, *V. pomeroyi*, *V. ponticus*, *V. proteolyticus*, *V. rotiferianus*, *V. ruber*, *V. rumoiensis*, *V. salmonicida*, *V. scophthalmi*, *V. splendidus*, *V. superstes*, *V. tapetis*, *V. tasmaniensis*, *V. tubiashii*, *V. vulnificus*, *V. wodanis*, and/or *V. xuii* or a combination of two or more thereof, or alternatively selected from the group consisting of *Vibrio alginolyticus*, *V. anguillarum*, *V. campbellii*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*, *V. penaeicida*, *V. vulnificus*, *V. nereis*, *V. tubiashi*, *V. fluvialis*, *V. splendidus*, and/or *V. nigripulchritudo* or a combination of two or more thereof. In a further aspect, the invertebrate vaccine and/or composition and/or formulation comprises dead and/or inactivated bacterial species of the genus *Paenibacillus* and/or dead and/or inactivated bacterial species of the genus *Vibrio*.

[0310] Laboratory tests can demonstrate the ability of treatment and immunization / vaccination of a female insect such as a mosquito or tick, e.g., *A. aegypti*, *Aedes albopictus* or *Aedes polynesiensis* using a dead and/or inactivated gram positive bacteria or cell wall fragments thereof by administering to the insect, e.g., such as a mosquito or tick, e.g., *A. aegypti* prevent or lower the infection rate in a population after blood feeding of the next generation population with disease- infected blood, e.g., Zika infected blood.

[0311] Adult female mosquitoes (Higgs WT control and TZIKV-C) or ticks are divided into groups: a) vaccinated, b) positive control, c) placebo control, and d) negative control.

[0312] Rearing of next generation mosquitos or ticks of each group is carries using methods described by Masters SW et al (2020) Rearing *Aedes aegypti* Mosquitoes in a Laboratory

Setting Centers for Disease Control and Prevention (.gov) <https://stacks.cdc.gov> > cdc > cdc_96121_DS1 and as modified for the growth of tick populations.

[0313] Briefly, F1 mosquitos or ticks of group a), b) c) are infected with the pathogen, e.g. ZIKV (FSS13025, Cambodia 2010 strain; GenBank accession no. JN860885) via membrane blood feeding (JT Ladner, et al., Complete genome sequences of five Zika virus isolates. *Genome Announc* 4, e00377-16 (2016). Controls receive non-infected, e.g., non-Zika infected blood. Four and fourteen days post feeding, midguts from blood-fed mosquitoes or ticks are dissected and ZIKV RNA copies are measured using real-time RT-qPCR. The experiments are carries out in three replicates.

[0314] To determine viral transmission, saliva from individual mosquitoes or ticks is collected at 14 dpi and ZIKV titers were measured using a median tissue culture infectious dose assay.

[0315] Thus, the use of oral administration via blood feeding of inactivated gram-positive bacteria to female insects, e.g., mosquitos or ticks can be an effective method to lower the ability to replication pathogenic viruses or bacteria, e.g., Zika viruses in the next generation of the disease-carrying insect.

Embodiments

[0316] A method for one or more of: vaccinating, treating, or immunizing an invertebrate, a population of invertebrates, its progeny, or the invertebrate host against a bacterial or viral infection or bacterial or viral disease, the method comprising administering an effective amount of dead and/or inactivated non-disease causing gram-positive bacteria or a cell wall fragment thereof to the invertebrate or the population of invertebrates, thereby vaccinating, treating or immunizing the invertebrate against the bacterial or viral disease or infection, optionally wherein the invertebrate is a crustacean or an insect.

[0317] The method of embodiment 1, further comprising administering an effective amount of a dead and/or inactivated gram negative bacteria or a cell wall fragment thereof to the invertebrate or the invertebrate population, and optionally wherein the invertebrate is a crustacean or an insect.

[0318] 3. The method of embodiment 1 or 2, wherein the bacterial or viral infection is caused by a pathogen of a single row of any of **Tables 1-4** or as shown in **Experiment No. 4**, and the dead and/or inactivated non-disease causing gram positive bacteria is identified in the corresponding row of Tables 1-4 or as shown in **Experiment No. 4**.

[0319] 4. The method of any one of embodiments 1-3, wherein the invertebrate is an insect and/or the invertebrate population is an insect or insect population or colony and the virus causing the viral disease or infection is selected from Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus, and optionally wherein the insect is a bee and/or the insect population is a bee population.

[0320] 5. The method of any one of embodiments 1-4, wherein the insect is a honey bee or a bumble bee or the population is a honey bee or a bumble bee population or colony and wherein the viral disease or infection is caused by Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus.

[0321] 6. The method of embodiment 5, wherein the the virus causing the disease or infection is Deformed Wing Virus-B (DWV-B).

[0322] 7. The method of any one of embodiments 1-6, wherein the dead and/or inactivated non-disease causing bacteria or cell wall fragment thereof is administered in combination with a carrier, optionally wherein the insect is a honey bee or a bumble bee and/or the population is a honey bee population or bumble bee population, the carrier comprises a bee food or a bee feed.

[0323] 8. The method of any one of embodiments 1-7, wherein the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated bacterial species of the genus *Paenibacillus*.

[0324] 9. The method of embodiment 8, wherein the dead and/or inactivated bacterial species is selected from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericamus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvifaciens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynnii*, and/or *P. xylanilyticus* or a combination of two or more thereof or optionally selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvens*, or a combination of two or more thereof.

[0325] 10. The method of any one of embodiments 1-9, wherein the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof.

[0326] 11. The method of any of embodiments 1-10, wherein the effective amount of dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises from

about 1.5×10^4 or 1.5×10^7 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments per gram of or carrier.

[0327] 12. The method of embodiment 11, wherein insects are administered from about 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments per dose.

[0328] 13. The method of embodiment 12, wherein the bee or population of bees are administered at least one, or at least two, or at least three, or at least four, or at least five, or at least six, or at least seven, or at least eight, or at least nine, or at least 10 dose(s) of from 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments.

[0329] 14. A method for one or more of: vaccinating, treating, or immunizing a bee, a bee progeny, or a population of bees against a viral infection or viral disease comprising administering an effective amount of dead and/or inactivated gram-positive bacteria or cell wall fragments thereof and optionally a carrier to the bee, the queen bee, or the population of bees, thereby vaccinating, treating or immunizing the bee, the progeny or population against the viral disease or infection.

[0330] 15. The method of embodiment 14, wherein the bee is a honey bee or population of bees is a honey bee population or honey bee colony and the virus causing the viral disease or infection is selected from Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus.

[0331] 16. The method of embodiment 14, wherein the bee is a bumble bee or a bumble bee population or colony and wherein the viral disease or infection is caused by Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus.

[0332] 17. The method of embodiment 14, wherein the bee is a honey bee and the population is a honey bee population or colony, and the virus causing the disease or infection is Deformed Wing Virus-B (DWV-B).

[0333] 18. The method of any one of embodiments 14-17, wherein the carrier comprises a bee food or a bee feed.

[0334] 19. The method of any one of embodiments 14-18, wherein the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated bacterial species of the genus *Paenibacillus*.

[0335] 20. The method of embodiment 19, wherein the dead and/or inactivated bacterial species is selected from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericanus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvifaciens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynnii*, and/or i or a combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus*

popilliae, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvens*, or a combination of two or more thereof.

[0336] 21. The method of any one of embodiments 14-19, wherein the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof.

[0337] 22. The method of any of embodiments 14-21, wherein the effective amount of dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises from about 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments per gram of or carrier.

[0338] 23. The method of embodiment 21, wherein bee or population of bees are administered from about 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments per dose.

[0339] 24. The method of embodiment 21, wherein the bee or population of bees are administered at least one, or at least two, or at least three, or at least four, or at least five, or at least six, or at least seven, or at least eight, or at least nine, or at least 10 dose(s) of from 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments.

[0340] 25. A method for one or more of treating or immunizing a bee population against a viral infection or disease infected with the virus causing the viral infection or disease, the method comprising introducing an uninfected queen bee to the bee population and administering an effective amount of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments and a carrier to the queen bee, thereby treating or immunizing the bee population against the viral disease or infection.

[0341] 26. The method of embodiment 25, wherein the bee population is a honey bee population or colony and the virus causing the viral disease or infection is selected from Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee

Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus.

[0342] 27. The method of embodiment 25, wherein the population or colony is a bumble bee population or colony and wherein the viral disease or infection is caused by Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus.

[0343] 28. The method of embodiment 25, wherein the population is a honey bee population or colony, and the virus causing the disease or infection is Deformed Wing Virus-B (DWV-B).

[0344] 29. The method of any one of embodiments 25-28, wherein the carrier comprises a bee food.

[0345] 30. The method of any one of embodiments 25-29, wherein the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated bacterial species of the genus *Paenibacillus*.

[0346] 31. The method of embodiment 30, wherein the dead and/or inactivated bacterial species is selected from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginoliticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericanus*, *P. antarcticus*, *P. apicarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P.*

phyllosphaerae, *P. polymyxa*, *P. popilliae*, *P. pulvifaciens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynnii*, and/or *P. xylanilyticus* or a combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvans*, or a combination of two or more thereof.

[0347] 32. The method of any one of embodiments 25-31, wherein the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof.

[0348] 33. The method of any of embodiments 12-19, wherein the effective amount of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments comprises from about 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments per gram of bee food or carrier.

[0349] 34. The method of embodiment 33, wherein a queen bee, worker bees, nurse bees or the larvae in the population are administered from about 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments per dose.

[0350] 35. The method of embodiment 34, wherein a queen bee, worker bees, nurse bees or bee larvae in the population are administered at least one, or at least two, or at least three, or at least four, or at least five, or at least six, or at least seven, or at least eight, or at least

nine, or at least 10 dose(s) of 7 about 1.5×10 or about 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments.

[0351] 36. A composition comprising dead and/or inactivated whole cells or cell wall fragments of a gram-negative bacteria and/or a gram-positive bacteria, and a carrier, and optionally wherein the carrier is phosphate buffered saline or water.

[0352] 37. The composition of embodiment 36, wherein the gram-negative bacteria comprises, or consists essentially of, or consists of, a bacterial species of the genus *Vibrio*.

[0353] 38. The composition of embodiment 36 or 37, wherein the gram-negative bacteria is selected from *Vibrio alginolyticus*, *V. anguillarum*, *V. campbelli*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*, *V. penaeicida*, *V. vulnificus*, *V. nereis*, *V. tubiashi*, *V. fluvialis*, *V. splendidus*, or *V. nigripulchritudo*.

[0354] 39. The composition of any one of embodiments 36-38, wherein the gram-positive bacteria comprises, or consists essentially of, or consists of a bacterial species of the genus *Paenibacillus*.

[0355] 40. The composition of any one of embodiments 36-39, wherein the gram-positive bacteria is selected from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericanus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdolanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvifaciens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wymii*, and/or *P. xylanilyticus* or a

combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvans*, or a combination of two or more thereof.

[0356] 41. The composition of any one of embodiments 36-40, wherein the carrier comprises a feed for an invertebrate.

[0357] 42. The composition of embodiment 41, wherein the invertebrate is a Crustacean.

[0358] 43. A composition of embodiment 41, where the invertebrate is a shrimp and the carrier is selected from one or more of phosphate buffered saline, water, a shrimp food or a shrimp feed.

[0359] 44. The composition of embodiment 41, wherein the invertebrate is identified in **Tables 1-4** or **Experiment No. 4**.

[0360] 45. The composition of embodiment 41, wherein the invertebrate is a shrimp and the carrier is a shrimp food or feed.

[0361] 46. The composition of any of embodiments 41-45, wherein the composition is formulated for oral administration, administration by injection, administration by immersion, or a combination of any two or more thereof.

[0362] 47. A method for one or more of: vaccinating, treating, or immunizing an invertebrate or its progeny against an infection or a disease caused by a viral or bacterial infection comprising administering an effective amount of the composition of any one of

embodiments of 41-46 to the invertebrate, thereby vaccinating, treating, or immunizing the invertebrate or its progeny against the viral or bacterial disease or infection in the animal.

[0363] 48. The method of embodiment 47, wherein the invertebrate is identified in **Tables 1-4** or **Experiment No. 4**.

[0364] 49. The method of embodiment 48, wherein the bacteria comprise, or consist essentially of, or consist of *P. larvae* and *P. thiaminolyticus*, the insect is a black soldier fly and the pathogen is *Hermetia illucens totivirus*.

[0365] 50. The method of embodiment 1 or 2, wherein the bacterial or viral infection is caused by a vector-borne pathogen as shown in **Table 2**, and the dead and/or inactivated non-disease causing gram positive bacteria is identified in the corresponding row of **Table 2** and the insect is as shown in the corresponding row of **Table 2**.

[0366] 51. The method of any one of embodiments 1, 2 or 50, wherein the insect is a Culicidae species, optionally the subfamilies Culicinae and Anophelinae, and further optionally wherein the Culicidae species is selected from *Aedes albopictus*, *Aedes aegypti*, or *Aedes polynesiensis*.

[0367] 52. A method for methods for one or more of: vaccinating, treating, preventing or immunizing an insect harboring a vector-borne pathogen against a viral infection, bacterial infection, bacterial disease, viral disease, and/or transmission of the disease, the method comprising administering an effective amount of dead and/or inactivated gram-positive bacteria or cell wall fragments thereof, optionally combined with a carrier, to the insect or insect host, thereby vaccinating, treating or immunizing the insect or the insect host against the viral or bacterial disease or infection, optionally wherein the insect is a mosquito or tick, and further optionally wherein the insect is a mosquito.

[0368] 53. The method of embodiment 52, wherein the insect or tick is identified in **Table 2**, optionally wherein the insect is a mosquito selected from the Culicinae or Anophelinae subfamilies, and further optionally wherein the Culicidae is selected from *Aedes albopictus*, *Aedes aegypti*, or *Aedes polynesiensis*.

[0369] 54. The method of any one of embodiments 50-53, wherein the gram-positive bacteria or cell wall fragment thereof is of the species *Paenibacillus*, optionally selected from one or more of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericamus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdianolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvificiens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynnii*, and/or *P. xylanilyticus*, and further optionally selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvens*, or a combination of two or more thereof.

[0370] 55. The method of any one of embodiments 50-54, wherein the gram-positive bacteria or a cell wall fragment thereof comprises or consists essentially of PL or the cell wall fragments thereof.

[0371] 56. The method of any one of embodiments 50-55, wherein the dead and/or inactivated gram positive bacteria is administered in combination with a carrier.

[0372] 57. The method of any of embodiments 50-56, wherein the effective amount of dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises from about 1.5×10^4 or 1.5×10^7 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments per gram of or carrier.

[0373] 58. The method of embodiment 57, wherein insects are administered from about 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments per dose.

[0374] 59. The method of embodiment 58, wherein the insect harboring a vector-borne illness is administered at least one, or at least two, or at least three, or at least four, or at least five, or at least six, or at least seven, or at least eight, or at least nine, or at least 10 dose(s) of from 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments thereof.

[0375] **Equivalents**

[0376] Unless otherwise defined, 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 technology belongs.

[0377] The present technology illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising,” “including,” “containing,” *etc.* shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the present technology claimed.

[0378] Thus, it should be understood that the materials, methods, and examples provided here are representative of preferred aspects, are exemplary, and are not intended as limitations on the scope of the present technology.

[0379] The present technology has been described broadly and generically herein. Each of the narrower species and sub-generic groupings falling within the generic disclosure also form part of the present technology. This includes the generic description of the present technology with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0380] In addition, where features or aspects of the present technology are described in terms of Markush groups, those skilled in the art will recognize that the present technology is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0381] All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety, to the same extent as if each were incorporated by reference individually. In case of conflict, the present specification, including definitions, will control.

[0382] Other aspects are set forth within the following claims.

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WHAT IS CLAIMED IS:

1. A method for one or more of: vaccinating, treating, or immunizing an invertebrate, a population of invertebrates, its progeny, or the invertebrate host against a bacterial or viral infection or bacterial or viral disease, the method comprising administering an effective amount of dead and/or inactivated non-disease causing gram-positive bacteria or a cell wall fragment thereof to the invertebrate or the population of invertebrates, thereby vaccinating, treating or immunizing the invertebrate against the bacterial or viral disease or infection, optionally wherein the invertebrate is a crustacean or an insect.
2. The method of claim 1, further comprising administering an effective amount of a dead and/or inactivated gram negative bacteria or a cell wall fragment thereof to the invertebrate or the invertebrate population, and optionally wherein the invertebrate is a crustacean or an insect.
3. The method of claim 1 or 2, wherein the bacterial or viral infection is caused by a pathogen of a single row of any of **Tables 1-4** or as shown in **Experiment No. 4**, and the dead and/or inactivated non-disease causing gram positive bacteria is identified in the corresponding row of Tables 1-4 or as shown in **Experiment No. 4**.
4. The method of claim 1 or 2, wherein the invertebrate is an insect and/or the invertebrate population is an insect or insect population or colony and the virus causing the viral disease or infection is selected from Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus, and optionally wherein the insect is a bee and/or the insect population is a bee population.
5. The method of claim 1 or 2, wherein the insect is a honey bee or a bumble bee or the population is a honey bee or a bumble bee population or colony and wherein the viral disease or infection is caused by Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus.

6. The method of claim 5, wherein the virus causing the disease or infection is Deformed Wing Virus-B (DWV-B).
7. The method of any claim 1 or 2, wherein the dead and/or inactivated non-disease causing bacteria or cell wall fragment thereof is administered in combination with a carrier, optionally wherein the insect is a honey bee or a bumble bee and/or the population is a honey bee population or bumble bee population, the carrier comprises a bee food or a bee feed.
8. The method of claim 1 or 2, wherein the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated bacterial species of the genus *Paenibacillus*.
9. The method of claim 8, wherein the dead and/or inactivated bacterial species is selected from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericamus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvifaciens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynnii*, and/or *P. xylanilyticus* or a combination of two or more thereof or optionally selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*,

Paenibacillus popilliae, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvens*, or a combination of two or more thereof.

10. The method of claim 1 or 2, wherein the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof.

11. The method of claim 1 or 2, wherein the effective amount of dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises from about 1.5×10^4 or 1.5×10^7 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments per gram of or carrier.

12. The method of claim 11, wherein insects are administered from about 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments per dose.

13. The method of claim 12, wherein the bee or population of bees are administered at least one, or at least two, or at least three, or at least four, or at least five, or at least six, or at least seven, or at least eight, or at least nine, or at least 10 dose(s) of from 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments.

14. A method for one or more of: vaccinating, treating, or immunizing a bee, a bee progeny, or a population of bees against a viral infection or viral disease comprising administering an effective amount of dead and/or inactivated gram-positive bacteria or cell wall fragments thereof and optionally a carrier to the bee, the queen bee, or the population of bees, thereby vaccinating, treating or immunizing the bee, the progeny or population against the viral disease or infection.

15. The method of claim 14, wherein the bee is a honey bee or population of bees is a honey bee population or honey bee colony and the virus causing the viral disease or infection is selected from Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow

Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus.

16. The method of claim 14, wherein the bee is a bumble bee or a bumble bee population or colony and wherein the viral disease or infection is caused by Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus.

17. The method of claim 14, wherein the bee is a honey bee and the population is a honey bee population or colony, and the virus causing the disease or infection is Deformed Wing Virus-B (DWV-B).

18. The method of claim 14 or 15, wherein the carrier comprises a bee food or a bee feed.

19. The method of claim 14 or 15, wherein the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated bacterial species of the genus *Paenibacillus*.

20. The method of claim 19, wherein the dead and/or inactivated bacterial species is selected from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericanus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azureducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvificiens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynnii*, and/or *P. xylanilyticus* or a

combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus macerans*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvens*, or a combination of two or more thereof.

21. The method of claim 14 or 15, wherein the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof.

22. The method of claim 14 or 15, wherein the effective amount of dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises from about 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments per gram of or carrier.

23. The method of claim 21, wherein bee or population of bees are administered from about 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments per dose.

24. The method of claim 21, wherein the bee or population of bees are administered at least one, or at least two, or at least three, or at least four, or at least five, or at least six, or at least seven, or at least eight, or at least nine, or at least 10 dose(s) of from 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments.

25. A method for one or more of treating or immunizing a bee population against a viral infection or disease infected with the virus causing the viral infection or disease, the method comprising introducing an uninfected queen bee to the bee population and administering an effective amount of dead and/or inactivated gram-positive bacteria whole cells or cell wall

fragments and a carrier to the queen bee, thereby treating or immunizing the bee population against the viral disease or infection.

26. The method of claim 25, wherein the bee population is a honey bee population or colony and the virus causing the viral disease or infection is selected from Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus.

27. The method of claim 25, wherein the population or colony is a bumble bee population or colony and wherein the viral disease or infection is caused by Deformed Wing Virus A, Deformed Wing Virus B, Deformed Wing Virus C, Acute Bee Paralysis virus, Israeli Acute Bee Paralysis Virus, Kashmir Bee Virus, Slow Bee Paralysis Virus, Lake Sinai Virus 1, Lake Sinai Virus 2, Chronic Bee Paralysis Virus, Sack brood Virus, Black Queen Cell Virus.

28. The method of claim 25, wherein the population is a honey bee population or colony, and the virus causing the disease or infection is Deformed Wing Virus-B (DWV-B).

29. The method of claim 25 or 26, wherein the carrier comprises a bee food.

30. The method of claim 25 or 26, wherein the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated bacterial species of the genus *Paenibacillus*.

31. The method of claim 30, wherein the dead and/or inactivated bacterial species is selected from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericanus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P.*

macquariensis, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvifaciens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynnii*, and/or *P. xylanilyticus* or a combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus maceran*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvans*, or a combination of two or more thereof.

32. The method of claim 25 or 26, wherein the dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises dead and/or inactivated *Paenibacillus larvae* (PL) or cell wall fragments thereof.

33. The method of claim 25 or 26, wherein the effective amount of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments comprises from about 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments per gram of bee food or carrier.

34. The method of claim 33, wherein a queen bee, worker bees, nurse bees or the larvae in the population are administered from about 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments per dose.

35. The method of claim 34, wherein a queen bee, worker bees, nurse bees or bee larvae in the population are administered at least one, or at least two, or at least three, or at least four, or at least five, or at least six, or at least seven, or at least eight, or at least nine, or at

least 10 dose(s) of 7 about 1.5×10 or about 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments.

36. A composition comprising dead and/or inactivated whole cells or cell wall fragments of a gram-negative bacteria and/or a gram-positive bacteria, and a carrier, optionally wherein the carrier is phosphate buffered saline or water.

37. The composition of claim 36, wherein the gram-negative bacteria comprises, or consists essentially of, or consists of, a bacterial species of the genus *Vibrio*.

38. The composition of claim 36 or 37, wherein the gram-negative bacteria is selected from *Vibrio alginolyticus*, *V. anguillarum*, *V. campbelli*, *V. damsela*, *V. harveyi*, *V. parahaemolyticus*, *V. penaeicida*, *V. vulnificus*, *V. nereis*, *V. tubiashi*, *V. fluvialis*, *V. splendidus*, or *V. nigrapulchritudo*.

39. The composition of claim 36 or 37, wherein the gram-positive bacteria comprises, or consists essentially of, or consists of a bacterial species of the genus *Paenibacillus*.

40. The composition of claim 36 or 37, wherein the gram-positive bacteria is selected from the group consisting of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericanus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdlanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P. glucanolyticus*, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamilae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvifaciens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynnii*, and/or *P. xylanilyticus* or a combination of two or more thereof or alternatively selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus*

doosanensis, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus macerans*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvans*, or a combination of two or more thereof.

41. The composition of claim 36 or 37, wherein the carrier comprises a feed for an invertebrate.
42. The composition of claim 41, wherein the invertebrate is a Crustacean.
43. A composition of claim 41, where the invertebrate is a shrimp and the feed is shrimp food.
44. The composition of claim 41, wherein the invertebrate is identified in **Tables 1-4** or **Experiment No. 4**.
45. The composition of claim 41, wherein the invertebrate is a shrimp and the carrier is selected from one or more of phosphate buffered saline, water, a shrimp food or a shrimp feed.
46. The composition of claim 41 or 42, wherein the composition is formulated for oral administration, administration by injection, administration by immersion, or a combination of any two or more thereof.
47. A method for one or more of: vaccinating, treating, or immunizing an invertebrate or its progeny against an infection or a disease caused by a viral or bacterial infection comprising administering an effective amount of the composition of any one of claims of 41-46 to the invertebrate, thereby vaccinating, treating or immunizing the invertebrate or its progeny against the viral or bacterial disease or infection in the animal.
48. The method of claim 47, wherein the invertebrate is identified in **Tables 1-4** or **Experiment No. 4**.

49. The method of claim 48, wherein the bacteria comprise, or consist essentially of, or consist of *P. larvae* and *P. thiaminolyticus*, the insect is a black soldier fly and the pathogen is *Hermetia illucens totivirus*.

50. The method of claim 1 or 2, wherein the bacterial or viral infection is caused by a vector-borne pathogen as shown in **Table 2**, and the dead and/or inactivated non-disease causing gram positive bacteria is identified in the corresponding row of **Table 2** and the insect is as shown in the corresponding row of **Table 2**.

51. The method of any one of claims 1, 2 or 50, wherein the insect is a Culicidae species, optionally the subfamilies Culicinae and Anophelinae, and further optionally wherein the Culicidae species is selected from *Aedes albopictus*, *Aedes aegypti*, or *Aedes polynesiensis*.

52. A method for methods for one or more of: vaccinating, treating, preventing or immunizing an insect harboring a vector-borne pathogen against a viral infection, bacterial infection, bacterial disease, viral disease, and/or transmission of the disease, the method comprising administering an effective amount of dead and/or inactivated gram-positive bacteria or cell wall fragments thereof, optionally combined with a carrier, to the insect or insect host, thereby vaccinating, treating or immunizing the insect or the insect host against the viral or bacterial disease or infection, optionally wherein the insect is a mosquito or tick, and further optionally wherein the insect is a mosquito.

53. The method of claim 52, wherein the insect or tick is identified in **Table 2**, optionally wherein the insect is a mosquito selected from the Culicinae or Anophelinae subfamilies, and further optionally wherein the Culicidae is selected from *Aedes albopictus*, *Aedes aegypti*, or *Aedes polynesiensis*.

54. The method of any one of claims 50-53, wherein the gram-positive bacteria or cell wall fragment thereof is of the species *Paenabacillus*, optionally selected from one or more of *P. agarexedens*, *P. agaridevorans*, *P. alginolyticus*, *P. alkaliterrae*, *P. alvei*, *P. amylolyticus*, *P. anaericamus*, *P. antarcticus*, *P. apiarius*, *P. assamensis*, *P. azoreducens*, *P. azotofixans*, *P. barcinonensis*, *P. borealis*, *P. brasiliensis*, *P. brassicae*[1], *P. campinasensis*, *P. chinjuensis*, *P. chitinolyticus*, *P. chondroitinus*, *P. cineris*, *P. cookii*, *P. curdalanolyticus*, *P. daejeonensis*, *P. dendritiformis*, *P. durum*, *P. ehimensis*, *P. elgii*, *P. favisporus*, *P.*

glucanolyticus, *P. glycanilyticus*, *P. gordonae*, *P. graminis*, *P. granivorans*, *P. hodogayensis*, *P. illinoisensis*, *P. jamaicae*, *P. kobensis*, *P. koleovorans*, *P. koreensis*, *P. kribbensis*, *P. lactis*, *P. larvae*, *P. lautus*, *P. lentimorbus*, *P. macerans*, *P. macquariensis*, *P. massiliensis*, *P. mendelii*, *P. motobuensis*, *P. naphthalenovorans*, *P. nematophilus*, *P. odorifer*, *P. pabuli*, *P. peoriae*, *P. phoenicis*, *P. phyllosphaerae*, *P. polymyxa*, *P. popilliae*, *P. pulvifaciens*, *P. rhizosphaerae*, *P. sanguinis*, *P. stellifer*, *Paenibacillus stellife*, *P. terrae*, *P. thiaminolyticus*, *P. timonensis*, *P. tundrae*, *P. turicensis*, *P. tylopili*, *P. validus*, *P. vortex*, *P. vulneris*, *P. wynnii*, and/or *P. xylanilyticus*, and further optionally selected from the group consisting of *Paenibacillus alvei*, *Paenibacillus dentritiformis*, *Paenibacillus amylolyticus*, *Paenibacillus campinasensis*, *Paenibacillus chondroitinus*, *Paenibacillus chungangensis*, *Paenibacillus doosanensis*, *Paenibacillus glucanolyticus*, *Paenibacillus humicus*, *Paenibacillus lactis*, *Paenibacillus larvae*, *Paenibacillus lautus*, *Paenibacillus lentimorbus*, *Paenibacillus macerans*, *Paenibacillus macerans-like*, *Paenibacillus macquariensis*, *Paenibacillus motobuensis*, *Paenibacillus pabuli*, *Paenibacillus phoenicis*, *Paenibacillus polymyxa*, *Paenibacillus popilliae*, *Paenibacillus puldeungensis*, *Paenibacillus residui*, *Paenibacillus stellife*, *Paenibacillus thiaminolyticus*, *Paenibacillus validus*, and *Paenibacillus xylanisolvans*, or a combination of two or more thereof.

55. The method of any one of claims 50-53, wherein the gram-positive bacteria or a cell wall fragment thereof comprises or consists essentially of PL or the cell wall fragments thereof.

56. The method of any one of claims 50-53, wherein the dead and/or inactivated gram positive bacteria is administered in combination with a carrier.

57. The method of any of claims 50-56, wherein the effective amount of dead and/or inactivated gram-positive bacteria or cell wall fragments thereof comprises from about 1.5×10^4 or 1.5×10^7 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments per gram of or carrier.

58. The method of claim 57, wherein insects are administered from about 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments per dose.

59. The method of claim 58, wherein the insect harboring a vector-borne illness is administered at least one, or at least two, or at least three, or at least four, or at least five, or at least six, or at least seven, or at least eight, or at least nine, or at least 10 dose(s) of from 1.5×10^7 or 1.5×10^4 to about 1.5×10^{11} antigen units of dead and/or inactivated gram-positive bacteria whole cells or cell wall fragments thereof.