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(54) Title: VACCINES AGAINST *MORITELLA VISCOSA*

(57) Abstract: The invention provides vaccines against emerging isolates of *M. viscosa* and methods of using said vaccines

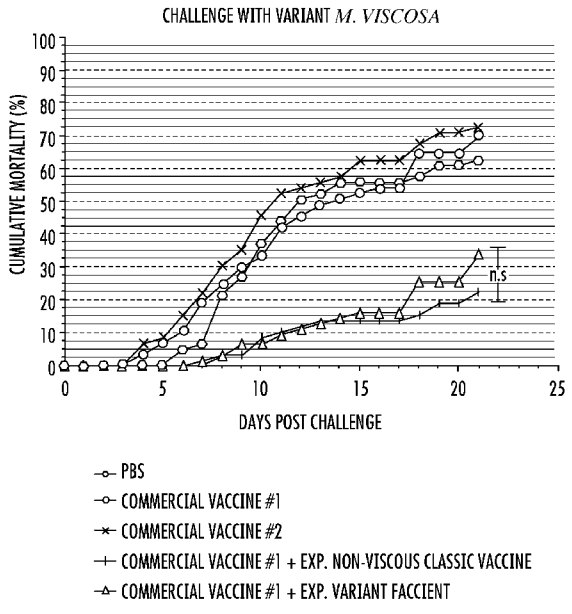

FIG. 3

WO 2023/225458 A1

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— *as to the identity of the inventor (Rule 4.17(i))*
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
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VACCINES AGAINST *MORITELLA VISCOSA*

FIELD OF THE INVENTION

[0001] This invention is generally in the field of aquaculture vaccines.

BACKGROUND

[0002] Winter ulcer disease affects both Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) and results in increased mortality rates as well as major economical losses due to downgrading of fish at slaughter.

[0003] *Moritella viscosa* (formally *Vibrio viscosus*) is the main aetiological agent of winter ulcer disease (Løvoll et al., 2009; Tunsjø et al., 2009; Björnsson et al., 2011; Karlsen et al., 2017a; Karlsen et al., 2017b). It is a gram-negative, psychrophilic, facultative anaerobic bacterium capable of both fermentative and respiratory metabolisms (Gudmundsdóttir and Björnsdóttir, 2007; Tunsjø et al., 2009; Björnsson et al., 2011). It is oxidase and catalase positive, requiring salt for growth; colonies are yellowish-translucent and generally viscous (Gudmundsdóttir and Björnsdóttir, 2007), although non-viscous M viscosa strains have also been isolated in the recent years.

[0004] Vaccines exist that protect against classic viscous strains of *M. viscosa*. However, current commercial vaccines are not effective against emerging strains that are both genotypically and/or phenotypically different from the classic strains. These emerging strains can be classified as variant, based on gyrB sequence, and classic non-viscous strains based on the non-viscous appearance after being cultured in agar plates, in contrast to the classic viscous *M. viscosa* strains that present adherent colonies forming viscous threads when manipulated with a loop.

[0005] Accordingly, new vaccines and methods are needed to effectively protect against the emerging strains as well as classic viscous strains.

SUMMARY OF INVENTION

[0006] This disclosure addresses these and other needs by providing, in the firth aspect, a vaccine comprising an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a classic non-viscous *M. viscosa* strain, for use in protecting fish against infection caused by variant *M. viscosa*.

[0007] The vaccine according to the first aspect of the invention may be used in protecting fish against infection caused by variant *M. viscosa* and classic non-viscous *M. viscosa*.

[0008] Also disclosed is a vaccine according to the first aspect of the invention, wherein the antigenic component consists essentially of, or consists of, the antigen derived from the antigen derived from the classic non-viscous *M. viscosa* strain.

[0009] Also disclosed is a vaccine according to the first aspect of the invention, wherein the antigenic component consists essentially of, or consists of, the antigen derived from the classic non-viscous *M. viscosa* strain and, optionally, an antigen derived from a classic viscous *M. viscosa* strain.

[0010] The vaccine according to the first aspect of the invention may be co-administered with a second vaccine, wherein the second vaccine of this first aspect comprises an antigen derived from a classic viscous *M. viscosa* strain. Preferably, the second vaccine does not contain an antigen derived from a variant strain of *M. viscosa*.

[0011] In the vaccine according to the first aspect of the invention, the antigen derived from the classic non-viscous *M. viscosa* strain is an inactivated preparation of the classic non-viscous *M. viscosa* strain. If the antigen derived from the classic viscous *M. viscosa* strain is present in the vaccine according to the first aspect, or in the second vaccine of the first aspect, said antigen may be in the form of an inactivated preparation of the classic viscous *M. viscosa* strain.

[0012] In the second aspect, this disclosure provides a vaccine comprising an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a variant *M. viscosa* strain, for use in protecting fish against infection caused by classic non-viscous *M. viscosa*.

[0013] The vaccine according to the second aspect of the invention may be used in protecting fish against infection caused by variant *M. viscosa* and classic non-viscous *M. viscosa*.

[0014] Also disclosed is a vaccine according to the second aspect of the invention, wherein the antigenic component consists essentially of, or consists of, the antigen derived from the antigen derived from the variant *M. viscosa* strain.

[0015] Also disclosed is a vaccine according to the second aspect of the invention, wherein the antigenic component consists essentially of, or consists of, the antigen derived from the variant *M. viscosa* strain and, optionally, an antigen derived from a classic viscous *M. viscosa* strain.

[0016] The vaccine according to the second aspect of the invention may be co-administered with a second vaccine of the second aspect, wherein the second vaccine comprises an antigen derived from a classic viscous *M. viscosa* strain. Preferably, the second vaccine of the second aspect does not contain an antigen derived from a classic non-viscous strain of *M. viscosa*.

[0017] Also disclosed in this second aspect is a vaccine wherein the antigen derived from the variant *M. viscosa* strain is an inactivated preparation of the variant *M. viscosa* strain. If the antigen derived from the classic viscous *M. viscosa* strain is present in the vaccine according to the second aspect, or in the second vaccine of the second aspect, said antigen may be in the form of an inactivated preparation of the classic viscous *M. viscosa* strain.

[0018] In the third aspect, this disclosure provides a vaccine comprising an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a variant *M. viscosa* strain, for use in protecting fish against infection caused by a classic non-viscous *M. viscosa* and classic viscous *M. viscosa.*

[0019] The vaccine according to this third aspect may be used in protecting fish against infection caused by variant *M. viscosa*, classic non-viscous *M. viscosa* and classic viscous *M. viscosa.*

[0020] The vaccine according to this third aspect of the invention does not include an antigen derived from a classic non-viscous *M. viscosa* strain and does not include an antigen derived from a classic viscous *M. viscosa* strain. In certain embodiments, the *M. viscosa* component of the vaccine according to this third aspect of the invention consists essentially or consists of the antigen derived from the variant *M. viscosa* strain.

[0021] In the vaccines according to this third aspect of the invention, the antigen derived from the variant *M. viscosa* strain is an inactivated preparation of said variant *M. viscosa* strain.

[0022] In the fourth aspect, the disclosure provides a vaccine comprising an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a classic non-viscous *M. viscosa* strain, for use in protecting fish against infection caused by variant *M. viscosa* and classic viscous *M. viscosa*.

[0023] The vaccine according to this fourth aspect may be used in protecting fish against infection caused by variant *M. viscosa*, classic non-viscous *M. viscosa* and classic viscous *M. viscosa.*

[0024] The vaccine according to this fourth aspect of the invention does not include an antigen derived from a variant *M. viscosa* strain and does not include an antigen derived from a classic viscous *M. viscosa* strain. In certain embodiment, the *M. viscosa* component of the vaccine according to this fourth aspect of the invention consists essentially or consists of the antigen derived from the classic non-viscous *M. viscosa* strain.

[0025] In the vaccines according to this fourth aspect of the invention, the antigen derived from the classic non-viscous *M. viscosa* strain is an inactivated preparation of said classic non-viscous *M. viscosa* strain.

[0026] The compositions according to the first, the second, the third, and the fourth aspect of the invention further contain non-*M. viscosa* antigens. In certain embodiments, said one or more non-*M. viscosa* antigens are selected from the group consisting of IPNV, ISAV, SPDV, *Aeromonas salmonicida, Vibrio anguillarum O1, O2, Vibrio (Aliivibrio) salmonicida, Yersinia ruckeri* O1.

[0027] The compositions according to the first, the second, the third, and the fourth aspect of the invention are provided as water-in-oil emulsions.

[0028] Any compositions described above preferably are used in salmonids, most preferably, Atlantic salmon (*Salmo salar*). In certain embodiments, the weight of said fish is about 15-200 grams at the time of vaccination.

[0029] The compositions disclosed herein are suitable for protecting said fish against infection comprises a reduction or an elimination of at least one symptom of *M. viscosa*. In certain embodiments, the at least one symptom is mortality.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] Fig. 1 is a photograph of Western Blot demonstrating that different antibodies recognize classic viscous *M. viscosa* on one hand and classic non-viscous *M. viscosa* and variant *M. viscosa* on the other.

[0031] Fig. 2 illustrates the cumulative survival of fish vaccinated with classic viscous, classic non-viscous and variant *M. viscosa* after the challenge with classic non-viscous *M. viscosa.*

[0032] Fig. 3 illustrates the cumulative survival of fish vaccinated with classic viscous, classic non-viscous and variant *M. viscosa* after the challenge with variant *M. viscosa.*

[0033] Fig. 4 illustrates the cumulative survival of fish vaccinated with classic viscous and variant *M. viscosa* after the challenge with classic viscous *M. viscosa.*

DETAILED DESCRIPTION

[0034] For a better understanding of the invention, the following non-limiting definitions are provided:

[0035] The term "about" or "approximately," when used in connection with a measurable numerical variable, refers to the indicated value of the variable and to all values of the variable that are within the experimental error of the indicated value (e.g., within the 95% confidence interval for the mean) or within 10 percent of the indicated value, whichever is greater.

[0036] The term "antigenic *M. viscosa* component" refers to one or more antigens derived from *M. viscosa*, including classic viscous strains, classic non-viscous strains and variant strains.

[0037] "Antigen derived from" a pathogen, including classic *M. viscosa*, classic non-viscous *M. viscosa*, and variant *M. viscosa* refers to the inactivated preparation of the desired *M. viscosa* subtype, as well as whole-bacterial extract and fractions of the extract including without limitations membrane/ cell wall extract.

[0038] "Classic *M. viscosa*" also referred to as "viscous *M. viscosa*" or "classic viscous *M. viscosa*" refers to *M. viscosa* strains that form viscous adherent colonies when cultured on blood agar at 15°C for 48 hours, with NaCl concentration below 2.5%. *M. viscosa* usually forms greyish colonies. When the colonies are manipulated with the loop, the colonies of classic viscous *M. viscosa* form viscous mucous threads.

[0039] "Classic non-viscous *M. viscosa*" refers to *M. viscosa* strains classified as classic based on gyrB sequences but these strains do not form viscous adherent colonies when cultured on blood agar at 15°C for 48 hours, with NaCl concentration below 2.5%.

[0040] Classic isolates (both viscous and non-viscous) of *M. viscosa* have a conserved sequence in their respective gyrB genes. Accordingly, classic isolates are the isolates that contain, in their respective gyrB sequences, a subsequence that is at least 96% identical to SEQ ID NO: 1 (for example, at least 97% or at least 98% identical).

[0041] Two or more vaccines are "co-administered" if they are administered within 15 minutes of each other. Preferably, said two or more vaccines are administered within 10 minutes, or within 5 minutes, or within 4 minutes, or within 3 minutes, or within 2 minutes, or within 1 minutes of each other.

[0042] "*M. viscosa*" which is not preceded by classic, or variant, or non-viscous encompasses all three subtypes of *M. viscosa*.

[0043] The term "pharmaceutically acceptable" refers to substances, which are within the scope of sound medical judgment, suitable for use in contact with the tissues of subjects without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit-to-risk ratio, and effective for their intended use.

[0044] The term "subject" refers to fish for which the administration of an adjuvant composition is desired.

[0045] The phrase "therapeutically effective amount" refers to an amount of an antigen or vaccine that would induce an immune response in a subject receiving the antigen or vaccine which is adequate to prevent or reduce signs or symptoms of disease, including adverse health effects or complications thereof, caused by infection with a pathogen, such as a virus or a bacterium. Humoral immunity or cell-mediated immunity or both humoral and cell-mediated immunity may be induced. The immunogenic response to a vaccine may be evaluated indirectly through measurement of antibody titers, lymphocyte proliferation assays, or directly through monitoring signs and symptoms after challenge with wild type strain. The protective immunity conferred by a vaccine can also be evaluated by measuring reduction in clinical signs such as mortality, morbidity, overall physical condition, and overall health and performance of the subject.

[0046] The term "treating" refers to preventing a disorder, condition, or disease to which such term applies, or to preventing or reducing one or more symptoms of such disorder, condition, or disease.

[0047] The term "treatment" refers to the act of "treating" as defined above.

[0048] The term "vaccine" refers to a composition that elicits protective immunity in the subject.

[0049] "Protecting against infection caused by *M. viscosa*" refers to reduction or elimination of at least one clinical sign caused by *M. viscosa*. Said clinical signs include skin ulcers that may be followed by terminal septicemia and the combination thereof. In a particularly preferred embodiment, the protection against infection caused by *M. viscosa* refers to reduction in mortality rates caused by *M. viscosa*.

[0050] "Variant *M. viscosa*": as noted previously, it has been determined that classic isolates (both viscous and non-viscous) of *M. viscosa* have a conserved sequence in their respective gyrB genes. Accordingly, classic isolates are the isolates that contain, in their respective gyrB sequences, a subsequence that is at least 98% identical to SEQ ID NO: 1. Conversely, in variant *M. viscosa* isolates, the respective subsequences of the gyrB sequences are less than 98% identical to SEQ ID NO: 1. Preferably, in variant *M. viscosa* isolates, the respective subsequences are 70-98% identical to SEQ ID NO: 1. In addition to the differences in gyrB sequences, for the purpose of this application, variant *M. viscosa* isolates are not recognized by antibodies raised in salmon against classic viscous *M. viscosa* strains under conditions described in Example 1.

[0051] In certain embodiments, the variant *M. viscosa* isolates have the subsequence in their gyrB gene sequences that are at least 90% identical to SEQ ID NO: 2, preferably, at least 95% identical to SEQ ID NO: 2, provided that these subsequences are no more than 98% identical to SEQ ID NO: 1.

M. viscosa

[0052] The inventors have surprisingly discovered that the antigens from a variant strain of *M. viscosa* cross-protect against the challenge with a classic non-viscous strain of *M. viscosa* and vice versa: the antigens from a classic non-viscous strain of *M. viscosa* cross-protect against the challenge with a variant strain of *M. viscosa*.

[0053] Accordingly, in the first aspect this application provides a vaccine comprising an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a classic non-viscous *M. viscosa* strain, for use in protecting fish against infection caused by variant *M. viscosa*. Also disclosed is a vaccine comprising an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a classic non-viscous *M. viscosa* strain, for use in protecting fish against infection caused by variant *M. viscosa*, wherein

said vaccine does not contain any variant *M. viscosa* antigens. These vaccines may be used to protect against the infections caused by variant *M. viscosa* and classic non-viscous *M. viscosa*. In some of these vaccines, the antigenic *M. viscosa* component consists of the antigen derived from a classic non-viscous *M. viscosa* strain.

[0054] The vaccines according to this first aspect may be combined with an antigen derived from classic viscous *M. viscosa*. Such vaccines can be used against *M. viscosa* infections, including classic non-viscous *M. viscosa*, variant *M. viscosa*, and classic viscous *M. viscosa*.

[0055] Alternatively, the vaccines containing antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising (or consisting of, as described above) an antigen derived from a classic non-viscous *M. viscosa* strain may be co-administered with a vaccine containing an antigen derived from the classic viscous strain of *M. viscosa*. This combination of co-administered vaccines may be used for protecting fish in need thereof against infection caused by classic non-viscous *M. viscosa*, variant *M. viscosa*, and classic viscous *M. viscosa*.

[0056] The antigens derived from a classic non-viscous strain of *M. viscosa* may be provided in the form of an inactivated classic non-viscous *M. viscosa* preparation, such as inactivated whole organisms. Methods of bacterial inactivation are well known and include, without limitations, incubation with formalin, BEI and/or betapropiolactone (BPL). Alternatively, the antigens may be subunits, whole-cell extracts of classic non-viscous *M. viscosa*, or fractions thereof, including, without limitation, membrane fraction.

[0057] Similarly, antigens derived from a classic viscous strain of *M. viscosa* may be inactivated classic viscous *M. viscosa* preparation, such as inactivated whole organisms. Methods of bacterial inactivation are well known and include, without limitations, incubation with formalin, BEI and/or betapropiolactone (BPL). Alternatively, the antigens may be subunits, whole-cell extracts of classic viscous *M. viscosa*, or fractions thereof, including, without limitation, membrane fraction.

[0058] The antigens derived from a classic viscous strain of *M. viscosa* and/or a classic non-viscous strain of *M. viscosa* may be provided in the form of attenuated bacteria. Methods of making live attenuated bacteria are well known in the art and include, without limitation, culture passaging.

[0059] The dosage of antigenic classic non-viscous *M. viscosa* component and classic viscous *M. viscosa* component in the vaccine may vary. Thus, for example, one dose of the vaccine may contain at least $1x10^6$ cells/dose of classic non-viscous *M. viscosa* component. Without limitations, one dose may contain about $5x10^6$ cells/dose, about $1x10^7$ cells/dose, about $5x10^7$ cells/dose, about $1x10^8$ cells/dose, $3x10^8$ cells/dose, $5 x10^8$ cells/dose, $1x10^9$ cells/dose. The dose may also contain from $1x10^6$ cells/dose to $1x10^7$ cells/dose, or $5x10^6$ cells/dose to $5x10^7$ cells/dose, or $1x10^7$ cells/dose to $1x10^8$ cells/dose, or $5x10^7$ cells/dose to $5x10^8$ cells/dose, or $1x10^8$ cells/dose to $1x10^9$ cells/dose.

[0060] Similarly, the amount of antigenic classic viscous *M. viscosa* component present in one dose of the vaccine (whether the same vaccine as the vaccine containing said antigenic variant *M. viscosa* component or the second vaccine) may be at least $1x10^6$ cells/dose of classic viscous *M. viscosa* component. Thus, for example, one dose of the vaccine may contain at least $1x10^6$ cells/dose of classic viscous *M. viscosa* component. Without limitations, one dose may contain about $5x10^6$ cells/dose, about $1x10^7$ cells/dose, about $5x10^7$ cells/dose, about $1x10^8$ cells/dose, $3x10^8$ cells/dose, $5 x10^8$ cells/dose, $1x10^9$ cells/dose. The dose may also contain from $1x10^6$ cells/dose to $1x10^7$ cells/dose, or $5x10^6$ cells/dose to $5x10^7$ cells/dose, or $1x10^7$ cells/dose to $1x10^8$ cells/dose, or $5x10^7$ cells/dose to $5x10^8$ cells/dose, or $1x10^8$ cells/dose to $1x10^9$ cells/dose.

[0061] In the second aspect this application provides a vaccine comprising an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a variant *M. viscosa* strain, for use in protecting fish against infection caused by classic non-viscous *M. viscosa.* Also disclosed is a vaccine comprising an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a variant *M. viscosa* strain, for use in protecting fish against infection caused by classic non-viscous *M. viscosa*, wherein said vaccine does not contain any classic non-viscous *M. viscosa* antigens. These vaccines may be used to protect against the infections caused by classic non-viscous *M. viscosa* and variant *M. viscosa*. In some of these vaccines, the antigenic *M. viscosa* component consists of the antigen derived from a variant *M. viscosa* strain.

[0062] The vaccines according to this second aspect may be combined with an antigen derived from classic viscous *M. viscosa*. Such vaccines can be used against *M. viscosa* infections, including variant *M. viscosa*, classic non-viscous *M. viscosa*, and classic viscous *M. viscosa*.

[0063] Alternatively, the vaccines containing antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising (or consisting of, as described above) an antigen derived from a variant *M. viscosa* strain may be co-administered with a vaccine containing an antigen derived from the classic viscous strain of *M. viscosa*. This combination of co-administered vaccines may be used for protecting fish in need thereof against infection caused by variant *M. viscosa*, classic non-viscous *M. viscosa*, and classic viscous *M. viscosa*.

[0064] The antigens derived from a variant strain of *M. viscosa* may be inactivated variant *M. viscosa* preparation, such as inactivated whole organisms. Methods of bacterial inactivation are well known and include, without limitations, incubation with formalin, BEI and/or betapropiolactone (BPL). Alternatively, the antigens may be whole-cell extracts of variant *M. viscosa*, or fractions thereof, including, without limitation, membrane fraction.

[0065] Similarly, antigens derived from a classic viscous strain of *M. viscosa* may be an inactivated classic viscous *M. viscosa* preparation, such as inactivated whole organisms. Methods of bacterial inactivation are well known and include, without limitations, incubation with formalin, BEI and/or betapropiolactone (BPL). Alternatively, the antigens may be whole-cell extracts of classic viscous *M. viscosa*, or fractions thereof, including, without limitation, membrane fraction.

[0066] The antigens derived from classic viscous strain of *M. viscosa* and/or a variant strain of *M. viscosa* may be provided in the form of attenuated bacteria. Methods of making live attenuated bacteria are well known in the art and include, without limitation, culture passaging.

[0067] The dosage of antigenic variant *M. viscosa* component and classic viscous *M. viscosa* component in the vaccine may vary. Thus, for example, one dose of the vaccine may contain at least $1x10^6$ cells/dose of the variant *M. viscosa* component. Without limitations, one dose may contain about $5x10^6$ cells/dose, about $1x10^7$ cells/dose, about $5x10^7$ cells/dose, about $1x10^8$ cells/dose, $3x10^8$ cells/dose, 5 $x10^8$ cells/dose, $1x10^9$ cells/dose. The dose may also contain from $1x10^6$ cells/dose to $1x10^7$ cells/dose, or $5x10^6$ cells/dose to $5x10^7$ cells/dose, or $1x10^7$ cells/dose

to $1x10^8$ cells/dose, or $5x10^7$ cells/dose to $5x10^8$ cells/dose, or $1x10^8$ cells/dose to $1x10^9$ cells/dose.

[0068] Similarly, the amount of antigenic classic viscous *M. viscosa* component present in one dose of the vaccine (whether the same vaccine as the vaccine containing said antigenic classic non-viscous *M. viscosa* component or the second vaccine) may be at least $1x10^6$ cells/dose of classic viscous *M. viscosa* component. Thus, for example, one dose of the vaccine may contain at least $1x10^6$ cells/dose of classic viscous *M. viscosa* component. Without limitations, one dose may contain about $5x10^6$ cells/dose, about $1x10^7$ cells/dose, about $5x10^7$ cells/dose, about $1x10^8$ cells/dose, $3x10^8$ cells/dose, 5 $x10^8$ cells/dose, $1x10^9$ cells/dose. The dose may also contain from $1x10^6$ cells/dose to $1x10^7$ cells/dose, or $5x10^6$ cells/dose to $5x10^7$ cells/dose, or $1x10^7$ cells/dose to $1x10^8$ cells/dose, or $5x10^7$ cells/dose to $5x10^8$ cells/dose, or $1x10^8$ cells/dose to $1x10^9$ cells/dose.

[0069] The inventors have also surprisingly discovered that vaccination with variant *M. viscosa* antigen provides cross-protection against classic viscous *M. viscosa* challenge, but not the other way around (i.e., vaccination with the classic viscous *M. viscosa* antigen does not protect against variant *M. viscosa* challenge). Accordingly, in the third aspect, the invention provides a vaccine comprising an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a variant *M. viscosa* strain, for use in protecting fish against infection caused by classic viscous *M. viscosa.*

[0070] As discussed above, the antigen derived from a variant *M. viscosa* strain can also be used in protecting fish against infection caused by classic non-viscous *M. viscosa*. Accordingly, this disclosure also provides a vaccine an antigenic *M viscosa* component, said antigenic *M viscosa* component comprising an antigen derived from a variant *M viscosa* strain, for use in protecting fish against infection caused by classic viscous *M. viscosa* and against infection caused by classic non-viscous *M. viscosa* strains. Therefore, the disclosure also provides a vaccine an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a variant *M. viscosa* strain, for use in protecting fish against infection caused by classic viscous *M. viscosa* and against infection caused by classic non-viscous *M. viscosa* strains, wherein said

antigenic *M. viscosa* component lacks antigens derived from classic viscous and classic non-viscous *M. viscosa* strains.

[0071] In the fourth aspect, the invention provides a vaccine comprising an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a classic non-viscous *M. viscosa* strain, for use in protecting fish against infection caused by classic viscous *M. viscosa*.

[0072] As discussed above, the antigen derived from a classic non-viscous *M. viscosa* strain can also be used in protecting fish against infection caused by variant *M. viscosa*. Accordingly, this disclosure also provides a vaccine an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a classic non-viscous *M. viscosa* strain, for use in protecting fish against infection caused by classic viscous *M. viscosa* and against infection caused by variant *M. viscosa* strains. Therefore, the disclosure also provides a vaccine an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a classic non-viscous *M. viscosa* strain, for use in protecting fish against infection caused by classic viscous *M. viscosa* and against infection caused by variant *M. viscosa* strains, wherein said antigenic *M. viscosa* component lacks antigens derived from classic viscous and variant *M. viscosa* strains.

[0073] The dosages of classic viscous, classic non-viscous and variant *M. viscosa* antigens for the vaccines described in connection with the first and the second aspects are also applicable for the vaccines according to these third and the fourth aspects of invention.

Additional antigens

[0074] In all four aspects, the respective antigenic components of the vaccines (or the second vaccines) described herein may contain one or more additional, non-*M. viscosa* antigens as described below.

[0075] Such antigens may be derived from a bacterial source, from a viral source, from an additional parasitical source, and/or from a fungal source. These additional antigens may be inactivated organisms recited below, or the antigens may be derived from these organisms, including recombinantly prepared antigens.

[0076] Polyvalent vaccines containing antigens from typical fish pathogens other than *M. viscosa* are well known in the art and are already commercially available. In addition, representative isolates of relevant fish pathogens are available from various sources.

[0077] In particular embodiments of the invention said antigen from a bacterial source is selected from the group consisting of: live, attenuated or killed bacteria of the species Piscirickettsias sp. Aeromonas sp., Vibrio sp., Aliivibrio sp., Listonella sp., Tenacibaculum sp., Pasteurella sp., Photobacterium sp, Flavobacterium sp., Yersinia sp., Renibacterium sp., Streptococcus sp., Lactococcus sp., Leuconostoc sp., Bifidobacterium sp., Pediococcus sp., Brevibacterium sp., Edwarsiella sp., Francisella sp., Pseudomonas sp., Cytophaga sp., Nocardia sp., Mycobacterium sp., parts or subunits of these bacteria, and any combination hereof.

[0078] Isolates of such bacteria are available, e.g. from LGC Promochem/American Type Culture Collection ATCC repository and distribution center (ATCC) including strains of *A. salmonicida* (ATCC 33658), *V. salmonicida* (ATCC 43839), *V. anguillarum* serotype O1(ATCC 43305) and O2(ATCC 19264). In addition, cultures of *Piscirickettsias salmonis* have been deposited in the European Collection of Cell Culture (ECACC), Health Protection Agency, Porton Down, Salisbury, Wiltshire (UK), SP4 0JG UK on the 9 Jun. 2006 under the following accession numbers: 06050901, 06050902, 06050903 and 07032110.

[0079] Other specific embodiments pertain to a vaccine, wherein said antigenic material obtained from a viral source other than the fish virus as defined above is from a virus selected from the group consisting of: Viral Hemorrhagic Septicemia Virus (VHSV), Infectious Hematopoietic Necrosis virus (IHNV), Infectious Pancreatic Necrosis Virus (IPNV), , Infectious Salmon Anaemia virus (ISAV), Salmon pancreatic disease virus (SPDV), Iridovirus, Nodavirus, Piscine myocarditis virus (PMCV) and heart and skeletal muscle inflammation virus (HSMIV). These antigens may be included as modified live or inactivated organisms, as parts or subunits of any one of these viruses, as DNA vaccines, and/or combinations thereof. Representative species of such viruses are available to the skilled artisan, for instance from the following deposits: infectious pancreatic necrosis virus (IPNV, ATCC VR-1318, country of origin: unknown), Viral Hemorrhagic Septicemia Virus (VHSV, ATCC VR_1389, country of origin: Denmark); Infectious Hematopoietic Necrosis virus (IHNV, ATCC VR-1392, country of origin: USA)); Pancreatic Necrosis

Virus; Infectious Salmon Anaemia (ISA) virus (ATCC VR-1554, country of origin: Canada).Patent deposits have previously been made by the present applicant of the following viral species: Heart and Skeletal Muscle Infection Virus (HSMIV, patent deposit nr ECACC 04050401, country of origin: Norway).

[0080] In more specific embodiments, said antigenic material obtained from a viral source other than the fish virus as defined above is from the group consisting of: Glycoprotein of Viral Hemorrhagic Septicemia Virus (VHSV), nucleoprotein of Viral Hemorrhagic Septicemia Virus (VHSV), glycoprotein of Infectious Hematopoietic Necrosis virus (IHNV), nucleoprotein structural proteins of Infectious Pancreatic Necrosis Virus (IPNV), , antigenic fragments of any of one of these proteins and combinations hereof.

[0081] In other embodiments said antigenic material from an additional parasitic source is from a source selected from the Lepeophtheirus Sp., Caligus Sp., and Ichthyophthirius Sp, parts of any one of these parasites, and combinations thereof. In yet other embodiments said antigenic material is from a fungal source selected from the group consisting of Saprolegnia Sp., Branchiomyces sanguinis, Branchiomyces demigrans and Icthyophonus hoferi.

[0082] In certain embodiments, the additional antigens to be included into the vaccine of the invention and/or the second vaccine containing the classic viscous *M. viscosa* are selected form the group consisting of IPNV, ISAV, SPDV, *Aeromonas salmonicida, Vibrio anguillarum O1, O2, Vibrio (Aliivibrio) salmonicida, Yersinia ruckeri* O1.

[0083] In other embodiments, the additional antigens to be included into the vaccine of the invention and/or the second vaccine containing the classic viscous *M. viscosa* are selected form the group consisting of IPNV, *Aeromonas salmonicida*, *Vibrio anguillarum* serotype 1 and O2, and *Vibrio (Aliivibrio) salmonicida.*

Excipients and adjuvants

[0084] The vaccines of the invention may further comprise a suitable pharmaceutical carrier and/or an adjuvant. The pharmaceutical carriers can be sterile liquids, such as water or buffer solutions, such as saline solutions and aqueous dextrose and glycerol solution. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk,

glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. The formulation should suit the mode of administration.

[0085] The appropriate carrier is evident to those skilled in the art and will depend in large part upon the route of administration. Additional components that may be present in this invention are adjuvants, preservatives, surface active agents, chemical stabilizers, suspending or dispersing agents. Typically, stabilizers, adjuvants and preservatives are optimized to determine the best formulation for efficacy in the target subject.

[0086] In a currently preferred embodiment, the vaccine comprises an adjuvant. Suitable adjuvants include, without limitations, oil. The vaccines disclosed herein may be formulated as oil in water emulsions or, more preferably, water-in-oil emulsions. Other formulations, such as water-in-oil-in-water (W/O/W) may also be prepared. In addition, the vaccine may comprise one or more suitable surface-active compounds or emulsifiers, e.g. CREMOPHORE®, TWEEN® and SPAN®. Also adjuvants such as interleukin, CpG and glycoproteins may be used.

[0087] The vaccine may also comprise a "vehicle". A vehicle is a device to which the antigen adheres, without being covalently bound to it. Such vehicles are biodegradable nano/micro-particles or -capsules of PLGA (poly-lactide-co-glycolic acid), alginate or chitosan, liposomes, niosomes, micelles, multiple emulsions and macrosols, all known in the art. A special form of such a vehicle, in which the antigen is partially embedded in the vehicle, is the so-called ISCOM (European patents EP 109.942, EP 180.564 and EP 242.380.

[0088] In certain embodiments, the vaccine described herein is formulated as a water-in-oil emulsion. Preferably, the oil is a mineral oil.

[0089] The vaccines described herein may be administered to the salmonid by a variety of routes, including, without limitation, intraperitoneally, intramuscularly, orally, and by immersion. Preferably, the vaccine is administered by an injection in a microdose such that the volume of one dose is under 500 µl, or under 400 µl, or under 300 µl or under 200 µl or about 100 µl or under 100 µl, or about 50 µl or about 25 µl.

[0090] The vaccine disclosed herein may be used in protecting multiple salmonid species against an infection. Suitable salmonids include, without limitations, Atlantic salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch*), rainbow trout (*Oncorhynchus mykiss*), sockeye salmon (*Oncorhynchus nerka*), Chinook salmon (*Oncorhynchus tshawytscha*) and other species.

[0091] Salmonids of different ages (or weights) may be vaccinated according to the invention. In certain embodiments, the salmonid weighs between about 15 and about 200 grams at the time of vaccination. Thus, the weight of the salmonid at the time of the vaccination may be between about 25 and about 150 grams or between about 40 and about 110 grams or between about 50 and about 100 grams.

[0092] The invention will now be described in the following illustrative examples.

EXAMPLES

Example 1: variant and classic non-viscous *M. viscosa* are not recognized by a salmon polyclonal antibody raised against classic viscous *M. viscosa*.

Materials and methods Western blot:

[0093] Ten strains of *M. viscosa* were investigated using Western blot. Origin and year of isolation, gyrB variant or classic type as well as viscosity phenotype with regards to viscosity are listed in table 1. Isolate number also corresponds to lane number in the Western blot membranes.

Table 1. Overview of *M. viscosa* strains included in the study. The bacteria were not inactivated prior to sample preparation under reducing conditions.

Isolate number	Country	Species	Year	gyrB (classic or variant)[#]	Viscous (yes/no)*
1	Norway	Atlantic salmon	2018	variant	Yes
2	Norway	Atlantic salmon	2020	variant	Yes
3	Norway	Atlantic salmon	2001	classic	Yes
4	Norway	Atlantic salmon	2020	classic	Yes
5	Norway	Atlantic salmon	2021	classic	No
6	Norway	Atlantic salmon	2020	classic	No
7	Norway	Rainbow trout	2019	variant	Yes
8	Norway	Lumpfish	2019	variant	Yes
9	Canada	Atlantic salmon	2019	variant	Yes
10	Iceland	Atlantic salmon	2021	variant	Yes

*When grown on blood agar at 15°C for 48 hours at 2% NaCl.
[#] Adapted from Grove et al., *Dis Aquat Org*, 93: 51–61,2010.

Antibodies/conjugates used:

1) Monoclonal mouse anti-trout/salmon IgM antibody, clone 4C10
2) Polyclonal rabbit anti-mouse HRP conjugated (Cat. #P0260, Dako)
3) Precision Protein STREPTACTIN® HRP conjugated (Cat. #161-0380, Bio Rad)
4) Polyclonal salmon a- variant *M. viscosa* antibody -generated in course of the project
5) Polyclonal salmon a-classic *M. viscosa* antibody - generated in course of the project
6) Polyclonal rabbit anti-variant *M. viscosa* antibody - generated in course of the project
7) Polyclonal rabbit anti-classic *M. viscosa* antibody - generated in course of the project
8) Swine anti-rabbit HRP conjugated (Cat. #P0217, Dako)

Table 2. List of materials.

Materials
Tris-buffered Saline + Tween (TBST)
Tris-buffered Saline (TBS)
Sample buffer:
2x Laemmli Sample Buffer, Bio-Rad
2-Mercaptoethanol, Sigma-Aldrich (diluted 1:20 in 2x Laemmli Sample Buffer)
CLARITY™ Western ECL Substrate, Bio-Rad
PRECISION PLUS PROTEIN™ All Blue Standards, Bio-Rad
PRECISION PLUS PROTEIN™ Unstained Standards, Bio-Rad
CRITERION™ TGX Stain-Free Precast Gel 4-20%, Bio-Rad
TRANS-BLOT TURBO BLOT™, Bio-Rad
Skim milk powder, Millipore

Sample preparation:

[0094] Bacterial cultures were spread on blood agar plates for each strain. The plates were incubated at 15°C. After two days incubation, colonies from the agar plates were inoculated in 10 ml growth medium in 25 cm^2 cell flasks and incubated at 15°C and 100 rpm.

[0095] After incubation, OD was measured. 2x1 ml were centrifuged, the supernatant pipetted out.

[0096] The 10 bacterial pelleted samples were prepared for Western blot. All pellets were resolved in reducing sample buffer, incubated at 100°C for 10 minutes and frozen until further analyses. The day of SDS-PAGE the samples were diluted/normalized to obtain a theoretical OD 2 (calculated from OD at harvest) with freshly made sample buffer, before they were used.

SDS-PAGE and WB:

[0097] 10 µl of each sample were added to the lanes on the gel (6 µl in the molecular weight marker-lanes). Four parallel gels were run.

[0098] The gels were run for approx. 3 minutes at 250 V and then approximately 50 minutes at 160 V. Equal volume of PRECISION PLUS PROTEIN™ All Blue Standard and PRECISION PLUS PROTEIN™ Unstained Standard were combined (3-5 µl of each) and used as a molecular weight standard. Four gels were made to make four blots; two for treatment with salmon plasmas and

two with rabbit anti-*M. viscosa* antibodies. The gels were then activated for 45 seconds on the gel-doc, imaged and transferred to the TRANS-BLOT® TURBO™ Transfer Blotting System (BioRad), where a 7-minute turbo program was used. The blotted membranes were immediately transferred to a blocking-buffer (5% skimmed milk in TBST (TBSTM)). The blots were blocked over night at 2-6°C.

[0099] The blots were then incubated with antibodies as described in table 3 and 4. The STREPTACTIN® HRP was added together with the other HRP-conjugated antibodies for visualisation of the molecular weight standards. The blots were washed 3x10 minutes with TBST between each incubation (TBS was used in the last washing step before substrate incubation) before incubation with the CLARITY™ Western substrate for 5 minutes before exposure on the gel-doc imaging system (BioRad).

Table 3. Antibodies, dilutions and incubation times for blots incubated with salmon antibodies. RT = Room temperature

Antibody		Dilution	Incubation
Ab1	Blot 1: Polyclonal salmon a- variant M. viscosa antibody Blot 2: Polyclonal salmon a-classic M. viscosa antibody	1:2000	Overnight. 2-8°C.
Ab2	Monoclonal mouse anti-trout/salmon IgM antibody, clone 4C10	1:3500	1h, RT.
Ab3	Rabbit anti-mouse- HRP conjugate, DAKO StrepTactin -HRP conjugate, BioRad	1:1000 1:3000	1h, RT.

Table 4. Antibodies, dilutions and incubation times for blots incubated with rabbit antibodies. RT = Room temperature

Antibody		**Dilution**	**Incubation**
Ab1	Blot 3: Polyclonal rabbit anti-variant M. viscosa antibody Blot 4: Polyclonal rabbit anti-classic M. viscosa antibody	1:1000	2h, RT.
Ab2	Swine anti-rabbit -HRP conjugate, DAKO StrepTactin -HRP conjugate, BioRad	1:1000 1:3000	1h, RT.

[00100] The results of the experiment are illustrated in Fig. 1. This figure demonstrates that classic viscous *M. viscosa* and variant *M. viscosa* are recognized by different antibodies. More specifically, these results show that the antibody raised against classic viscous M viscosa in salmon does not recognize classic non-viscous *M. viscosa* and variant *M. viscosa*. Surprisingly, it was also demonstrated that classic non-viscous *M. viscosa* and variant *M. viscosa* are recognized by the same antibody.

Example 2: vaccination with variant *M. viscosa* cross-protects against classic non-viscous challenge, while vaccination with classic viscous *M. viscosa* does not

Materials and methods

[00101] Bath challenge studies were comparative, investigator-blinded, negative controlled and randomised laboratory experiments. The efficacy of different oil-adjuvanted injectable vaccines in protecting Atlantic salmon against experimental infection with different isolates of *M. viscosa* were investigated in the study. The experimental vaccines containing field isolates of classic non-viscous *M. viscosa* and variant *M. viscosa* adjuvanted with water-in-oil (W/O) emulsion were administered intraperitoneally by co-injection with commercial vaccines according to manufacturers' instructions. Both of these commercial vaccines contained classic viscous strains of *M. viscosa*. A negative control group was included. The fish was approximately 25 grams in average at vaccination.

[00102] The fish was exposed to continuous light (24:0) in order to smoltify prior to transfer and challenge in sea water. Onset of photomanipulation started approximately 6 weeks prior to challenge with a classic non-viscous strain of *M. viscosa*. Challenge was conducted by immersion 13 weeks post vaccination. Due to biomass considerations, the fish was challenged in two 500 L tanks per challenge isolate and the results from the identical replicate tanks was combined. A total of approximately 60 fish (30 fish/group per tank) per group was challenged per challenge isolate.

[00103] One week prior to challenge, the fish was transferred to the disease facility and equally distributed into two duplicate 500 L tanks per challenge isolate and adapted to 34‰ salinity seawater, 8ºC, 24:0 light regimen, which was the environmental parameters during the challenge period. The challenge material was cultivated fresh from a frozen bacterial stock, in shake flask

culture medium based on yeast extract at 12 degrees Celsius for two days with shaking. The fish was challenged by reducing the water volume in the tank before adding the challenge material directly into the tank.

[00104] Challenge was performed 13 weeks post vaccination. The fish were observed daily post challenge, and fish with ulcers were euthanized and recorded as mortalities in the mortality log. Efficacy was evaluated by statistical comparison of protection against mortality and ulceration between the vaccinated groups and the negative control group post challenge. The study fish were unvaccinated, free from clinical disease and had a valid health certificate. Injured or deformed fish were excluded from the study.

[00105] The results of the experiment are illustrated in Fig. 2.

[00106] As one can see, the group treated with PBS as well as the groups treated with commercial vaccines (both containing classic viscous *M. viscosa* antigen) resulted in almost 100% mortality eighteen days after the fish was challenged with a classic non-viscous *M. viscosa* strain, thus supporting the validity of the challenge model. There was no statistically significant difference between these three groups.

[00107] In contrast, groups vaccinated with compositions comprising either a classic non-viscous *M. viscosa* antigen or a variant antigen exhibited only about 70% mortality (no significant difference between these two groups but $p < 0.0001$ compared to the groups treated with PBS or commercial vaccines).

[00108] These results suggest that classic viscous *M. viscosa* antigens do not cross-protect against the challenge with classic non-viscous *M. viscosa* strains. Classic non-viscous antigens protect against the challenge with classic non-viscous *M. viscosa* strains. Surprisingly, variant *M. viscosa* antigens cross-protect against the challenge with classic non-viscous *M. viscosa* strains.

Example 3: vaccination classic non-viscous *M. viscosa* cross-protects against variant M. viscosa challenge, while vaccination with classic viscous *M. viscosa* does not

[00109] The materials and methods were the same as in Example 2, except a variant *M. viscosa* isolate was used as a challenge strain.

[00110] The results of the experiment are illustrated in Fig. 3.

[00111] As one can see, the group treated with PBS as well as the groups treated with commercial vaccines (both containing classic viscous *M. viscosa* antigen) resulted in about 60 to 70% mortality 22 days after the fish was challenged with a classic non-viscous *M. viscosa* strain, demonstrating that the challenge model was valid. There was no statistically significant difference between these three groups.

[00112] In contrast, groups vaccinated with compositions comprising either a classic non-viscous *M. viscosa* antigen or a variant antigen exhibited only about 20-35% mortality (no significant difference between these two groups but $p < 0.0001$ compared to the groups treated with PBS or commercial vaccines).

[00113] These results suggest that classic viscous *M. viscosa* antigens do not cross-protect against the challenge with variant *M. viscosa* strains. Variant antigens protect against the challenge with variant *M. viscosa* strains. Surprisingly, classic non-viscous *M. viscosa* antigens cross-protect against the challenge with variant *M. viscosa* strains.

Example 4: vaccination with variant *M. viscosa* protects against the challenge with classic viscous *M. viscosa*.

Materials and methods:

[00114] The cross-protective efficacy of the monovalent oil-adjuvanted (W/O emulsion) variant *M. viscosa* vaccine was assessed in an investigator-blinded, negative controlled and randomised laboratory experiment.

[00115] A monovalent variant *M. viscosa* vaccine was administered by intraperitoneal injection to one group. A second group was vaccinated with a vaccine containing classic viscous *M. viscosa* and a negative control group was injected with Phosphate Buffered Saline (PBS). The fish were kept in 500 L tanks at 15°C freshwater during the immunisation period, and were exposed to a continuous light regime (24:0 light:dark) for approximately 6 weeks in order to smoltify prior to bath challenge in seawater. The challenge material (a classic viscous *M. viscosa* isolate) was cultivated fresh from a frozen bacterial stock, in shake flask culture medium based on yeast extract at 12 degrees Celsius for two days with shaking. The fish was challenged by reducing the water volume in the tank before adding the challenge material directly into the tank.

[00116] Challenge was performed by immersion after an immunisation period for approximately 9 weeks. The challenge intended to investigate any cross-protective efficacy of the monovalent vaccine containing inactivated antigen of variant *M. viscosa* against a classic viscous *M. viscosa* isolate. Approximately one week prior to challenge, the fish were transferred to the disease facility and the groups were equally distributed into parallel 500 L tanks and gradually acclimatized to 8°C, 34‰ salinity seawater. In order to reduce biomass density at challenge, the groups were challenged in two duplicate tanks per challenge isolate, and the results from the two tanks were combined. The fish were observed daily post challenge, and fish with ulcers were euthanized and recorded as mortalities in the mortality log. Efficacy was evaluated by statistical comparison of protection against mortality and ulceration between the vaccinated groups and the negative control group post challenge. The study fish were unvaccinated, free from clinical disease and had a valid health certificate. Injured or deformed fish were excluded from the study.

[00117] The results are illustrated in Fig. 4. The group treated with PBS exhibited over 40% mortality 22 days after the challenge. In contrast, vaccination with a classic *M. viscosa* antigen or with a variant *M. viscosa* antigen resulted in a statistically significant drop in cumulative mortality, to about 10 and 75, respectively, by day 22 after the challenge. These results indicate that vaccination with a variant *M. viscosa* antigen can cross-protect against the challenge with a classic viscous *M. viscosa*. Given the similar responses of the groups vaccinated with variant *M. viscosa* antigens and the groups vaccinated with classic-non-viscous *M. viscosa* antigens, these results also strongly suggest that vaccination with a classic non-viscous *M. viscosa* antigen can cross-protect against the challenge with a classic *M. viscosa*.

[00118] All publications cited in the specification, both patent publications and non-patent publications, are indicative of the level of skill of those skilled in the art to which this invention pertains. All these publications are herein fully incorporated by reference to the same extent as if each individual publication were specifically and individually indicated as being incorporated by reference.

[00119] Although the invention herein has been described with reference to particular embodiments, it is to be understood that these embodiments are merely illustrative of the principles and applications of the present invention. It is therefore to be understood that

numerous modifications may be made to the illustrative embodiments and that other arrangements may be devised without departing from the spirit and scope of the present invention as defined by the following claims.

CLAIMS

1. A vaccine comprising an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a classic non-viscous *M. viscosa* strain, for use in protecting fish against infection caused by variant *M. viscosa*.

2. A vaccine of claim 1, for use in protecting fish against infection caused by variant *M. viscosa* and by classic non-viscous *M. viscosa*.

3. The vaccine of claim 1 or 2, wherein said vaccine does not include an antigen derived from a variant *M. viscosa* strain.

4. The vaccine of claim 3, wherein said antigenic *M. viscosa* component consists of the antigen derived from the classic non-viscous *M. viscosa* strain.

5. The vaccine of any one of claims 1-3, wherein said vaccine is co-administered with a second vaccine comprising an antigen derived from a classic viscous *M. viscosa* strain.

6. The vaccine of claim 5, wherein said second vaccine does not contain an antigen derived from a variant *M. viscosa* strain.

7. The vaccine of any one of claims 1-3, for use in protecting fish against infection caused by variant *M. viscosa*, classic viscous *M. viscosa*, and classic non-viscous *M. viscosa*, wherein said *M. viscosa* antigenic compound further comprises an antigen derived from a classic viscous *M. viscosa* strain.

8. The vaccine of claim 7, wherein the antigenic *M. viscosa* component consists of the antigen derived from the classic non-viscous *M. viscosa* strain and the antigen derived from the classic viscous *M. viscosa* strain.

9. The vaccine of any one of claims 1-8 wherein the antigen derived from the classic non-viscous *M. viscosa* strain is an inactivated preparation of the classic non-viscous *M. viscosa* strain.

10. A vaccine comprising an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a variant *M. viscosa* strain, for use in protecting fish against infection caused by classic non-viscous *M. viscosa*.

11. A vaccine of claim 10, for use in protecting fish against infection caused by variant *M. viscosa* and by classic non-viscous *M. viscosa*.

12. The vaccine of claim 10 or 11, wherein said vaccine does not include an antigen derived from a classic non-viscous *M. viscosa* strain.

13. The vaccine of claim 12, wherein said antigenic *M. viscosa* component consists of the antigen derived from the variant *M. viscosa* strain.

14. The vaccine of any one of claims 10-12, wherein said vaccine is co-administered with a second vaccine comprising an antigen derived from a classic viscous *M. viscosa* strain.

15. The vaccine of claim 14, wherein said second vaccine does not contain an antigen derived from a classic non-viscous *M. viscosa* strain.

16. The vaccine of any one of claims 11-13, for use in protecting fish against infection caused by variant *M. viscosa*, classic viscous *M. viscosa*, and classic non-viscous *M. viscosa*, wherein said *M. viscosa* antigenic compound further comprises an antigen derived from a classic viscous *M. viscosa* strain.

17. The vaccine of claim 16, wherein the antigenic *M. viscosa* component consists of the antigen derived from the variant *M. viscosa* strain and the antigen derived from the classic viscous *M. viscosa* strain.

18. The vaccine of any one of claims 10-17 wherein the antigen derived from the variant *M. viscosa* strain is an inactivated preparation of the variant *M. viscosa* strain.

19. The vaccine according to any one of claims 5-9 or 14-18, wherein the antigen derived from the classic *M. viscosa* strain is an inactivated preparation of said classic *M. viscosa* strain.

20. A vaccine comprising an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a variant *M. viscosa* strain, for use in protecting fish against infection caused by classic non-viscous *M. viscosa* and classic viscous *M. viscosa*.

21. A vaccine of claim 20, for use in protecting fish against infection caused by variant *M. viscosa*.

22. The vaccine according to claim 20 or claim 21, wherein said vaccine does not include an antigen derived from a classic non-viscous *M. viscosa* strain and said vaccine does not include an antigen derived from a classic viscous *M. viscosa* strain.

23. The vaccine according to any one of claims 20-22, wherein the antigenic *M. viscosa component* consists of the antigen derived from the variant *M. viscosa* strain.

24. The vaccine according to any one of claims 20-23, wherein the antigen derived from the variant *M. viscosa* strain is an inactivated preparation of said variant *M. viscosa* strain.

25. A vaccine comprising an antigenic *M. viscosa* component, said antigenic *M. viscosa* component comprising an antigen derived from a classic non-viscous *M. viscosa* strain, for use in protecting fish against infection caused by variant *M. viscosa* and classic viscous *M. viscosa*.

26. A vaccine of claim 25, for use in protecting fish against infection caused by classic non-viscous *M. viscosa*.

27. The vaccine according to claim 25 or claim 26, wherein said vaccine does not include an antigen derived from a variant *M. viscosa* strain and said vaccine does not include an antigen derived from a classic viscous *M. viscosa* strain.

28. The vaccine according to any one of claims 25-27, wherein the antigenic *M. viscosa* component consists of the antigen derived from the classic non-viscous *M. viscosa* strain.

29. The vaccine according to any one of claims 25-28, wherein the antigen derived from the classic non-viscous *M. viscosa* strain is an inactivated preparation of the variant *M. viscosa* strain.

30. The vaccine according to any one of claims 1-29, comprising one or more non *M. viscosa* antigens.

31. The vaccine of claim 30, wherein the second vaccine comprises one or more non *M. viscosa* antigens.

32. The vaccine of claim 30 or claim 31 wherein said one or more non *M. viscosa* antigens are selected from the group consisting of IPNV, ISAV, SPDV, *Aeromonas salmonicida, Vibrio anguillarum O1, O2, Vibrio (Aliivibrio) salmonicida, Yersinia ruckeri* O1.

33. The vaccine of any one of claims 1-32 wherein said vaccine is in a form of a water-in-oil emulsion.

34. The vaccine of any one of claims 1-33 wherein said fish is a salmonid.

35. The vaccine of claim 34 wherein said salmonid is Atlantic salmon (*Salmo salar*).

36. The vaccine of any one of claims 1-35 wherein said fish weighs 15-200 grams.

37. The vaccine of any one of claims 1-36 wherein said vaccine is administered peritoneally.

38. The vaccine according to any one of claims 1-37, wherein protecting said fish against infection comprises a reduction or an elimination of at least one symptom of *M. viscosa*.

39. The vaccine according to any one of claims 1-38, wherein protecting said fish against infection comprises a reduction of mortality caused by *M. viscosa*.

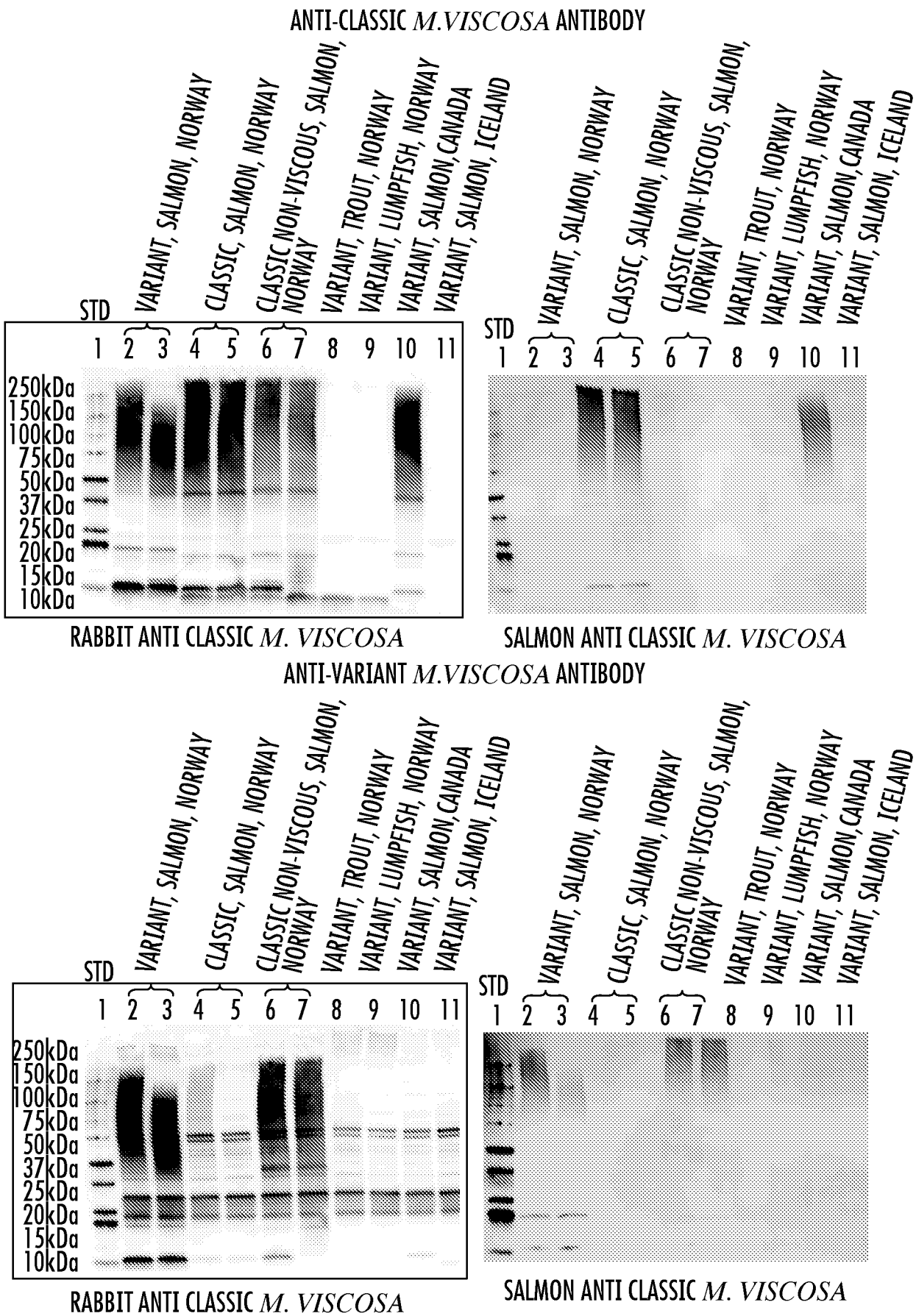
ANTI-CLASSIC M.VISCOSA ANTIBODY
STD
VARIANT, SALMON, NORWAY
CLASSIC, SALMON, NORWAY
CLASSIC NON-VISCOUS, SALMON, NORWAY
VARIANT, TROUT, NORWAY
VARIANT, LUMPFISH, NORWAY
VARIANT, SALMON, CANADA
VARIANT, SALMON, ICELAND
1 2 3 4 5 6 7 8 9 10 11
250kDa
150kDa
100kDa
75kDa
50kDa
37kDa
25kDa
20kDa
15kDa
10kDa
RABBIT ANTI CLASSIC M. VISCOSA
SALMON ANTI CLASSIC M. VISCOSA
ANTI-VARIANT M.VISCOSA ANTIBODY
STD
VARIANT, SALMON, NORWAY
CLASSIC, SALMON, NORWAY
CLASSIC NON-VISCOUS, SALMON, NORWAY
VARIANT, TROUT, NORWAY
VARIANT, LUMPFISH, NORWAY
VARIANT, SALMON, CANADA
VARIANT, SALMON, ICELAND
1 2 3 4 5 6 7 8 9 10 11
250kDa
150kDa
100kDa
75kDa
50kDa
37kDa
25kDa
20kDa
15kDa
10kDa
RABBIT ANTI CLASSIC M. VISCOSA
SALMON ANTI CLASSIC M. VISCOSA

FIG. 1

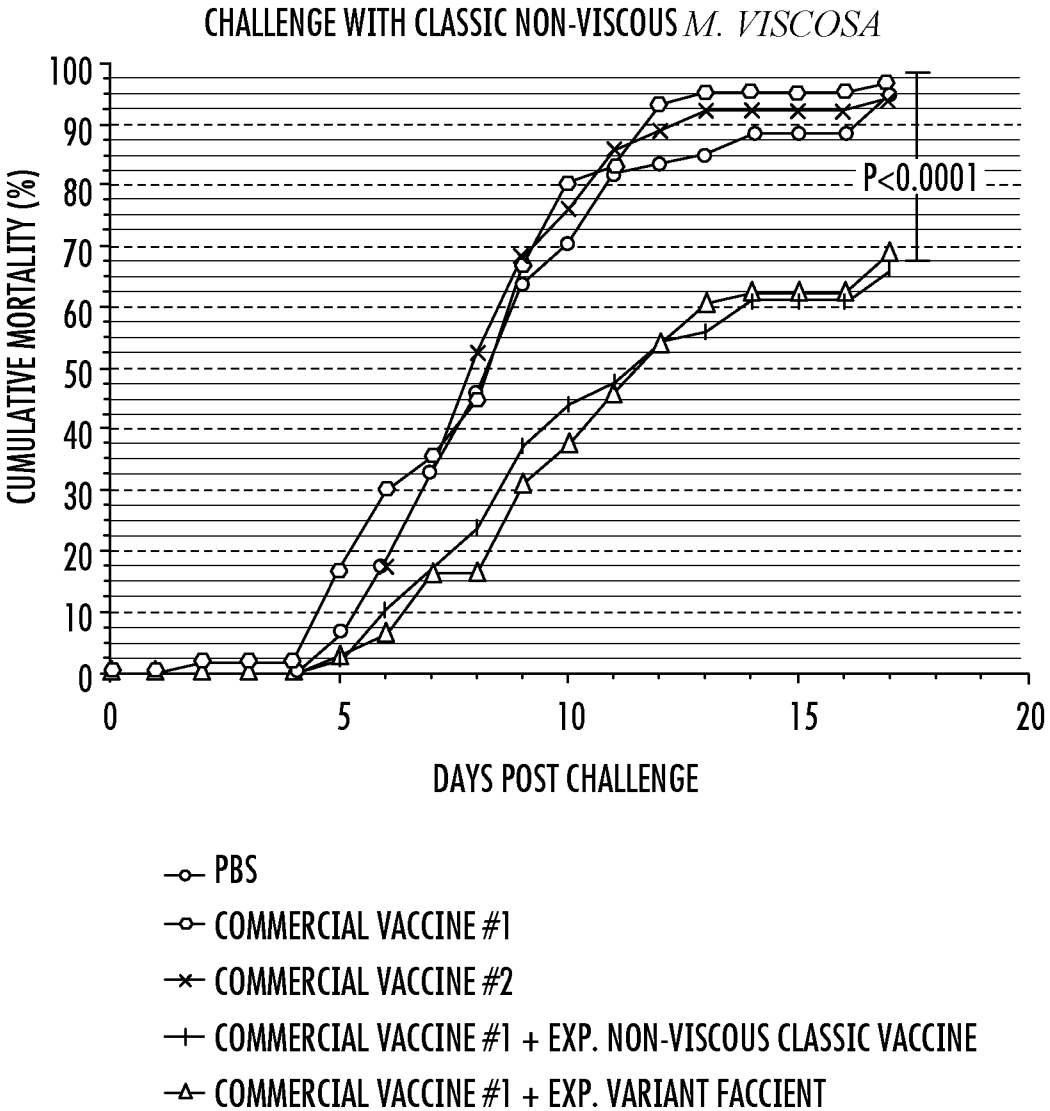
CHALLENGE WITH CLASSIC NON-VISCOUS M. VISCOSA
CUMULATIVE MORTALITY (%)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
DAYS POST CHALLENGE
P<0.0001
PBS
COMMERCIAL VACCINE #1
COMMERCIAL VACCINE #2
COMMERCIAL VACCINE #1 + EXP. NON-VISCOUS CLASSIC VACCINE
COMMERCIAL VACCINE #1 + EXP. VARIANT FACCIENT

FIG. 2

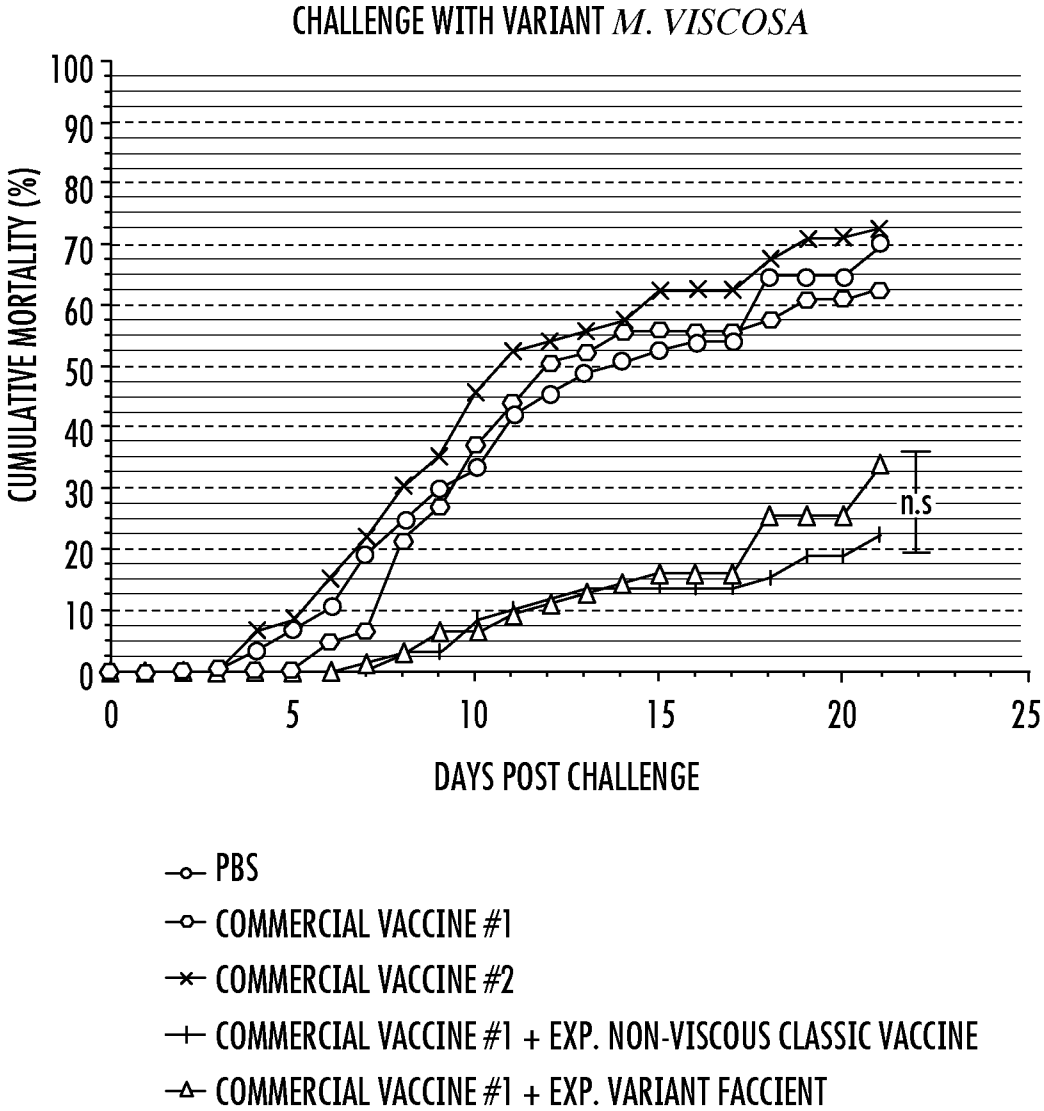
CHALLENGE WITH VARIANT M. VISCOSA
CUMULATIVE MORTALITY (%)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
DAYS POST CHALLENGE
n.s
PBS
COMMERCIAL VACCINE #1
COMMERCIAL VACCINE #2
COMMERCIAL VACCINE #1 + EXP. NON-VISCOUS CLASSIC VACCINE
COMMERCIAL VACCINE #1 + EXP. VARIANT FACCIENT

FIG. 3

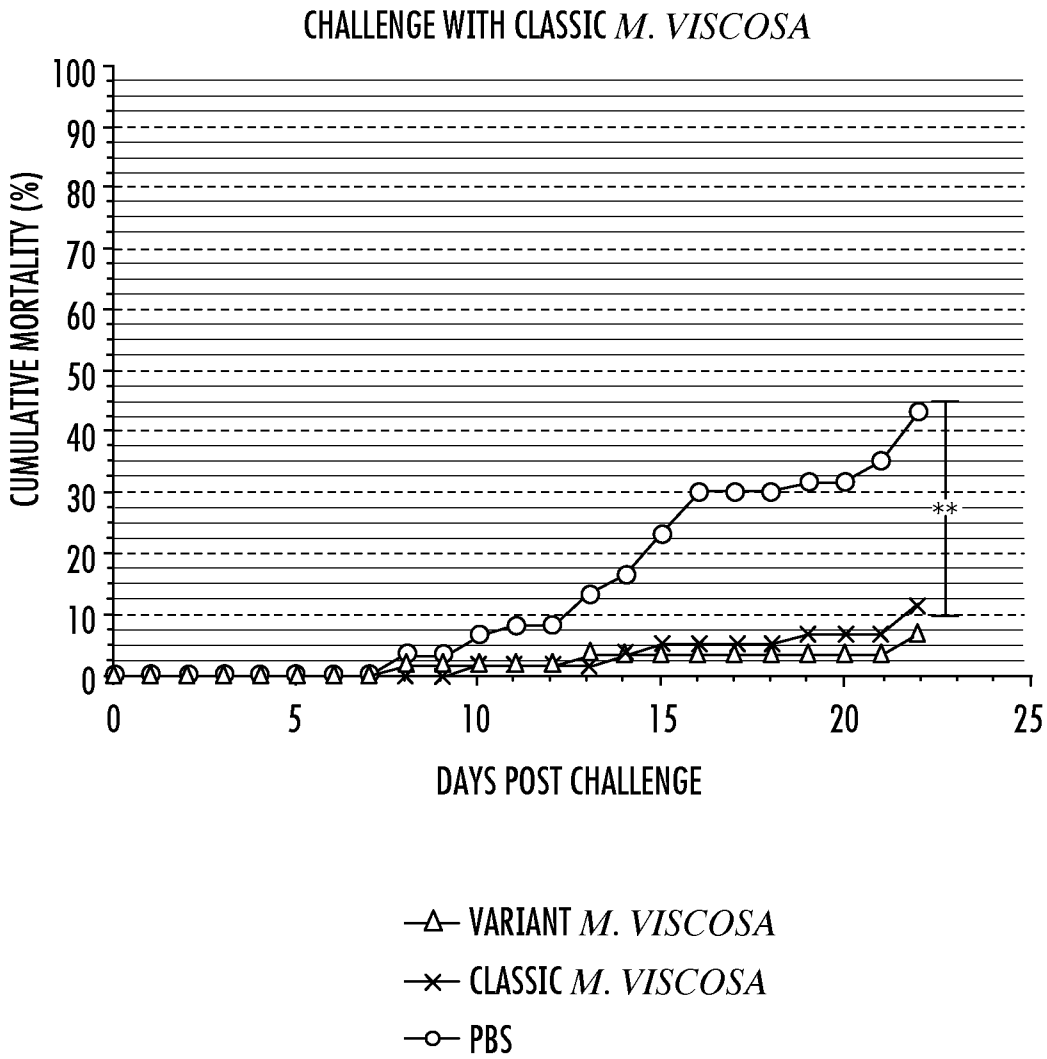
CHALLENGE WITH CLASSIC M. VISCOSA
CUMULATIVE MORTALITY (%)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
DAYS POST CHALLENGE
**
VARIANT M. VISCOSA
CLASSIC M. VISCOSA
PBS

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/066908

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P31/04 A61K39/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EINARSDOTTIR THORBJORG ET AL: "Moritella viscosa in lumpfish (Cyclopterus lumpus) and Atlantic salmon (Salmo salar)", JOURNAL OF FISH DISEASES, vol. 41, no. 11, 21 August 2018 (2018-08-21), pages 1751-1758, XP093077842, GB ISSN: 0140-7775, DOI: 10.1111/jfd.12884 Retrieved from the Internet: URL:https://api.wiley.com/onlinelibrary/tdm/v1/articles/10.1111%2Fjfd.12884> figures 1,2 abstract ----- -/--	1-39

[x] Further documents are listed in the continuation of Box C.
[ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 September 2023	11/09/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Covone-van Hees, M

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/066908

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KARLSEN CHRISTIAN ET AL: "Host specificity and clade dependent distribution of putative virulence genes inMoritella viscosa", MICROBIAL PATHOGENESIS, ACADEMIC PRESS LIMITED, NEW YORK, NY, US, vol. 77, 29 September 2014 (2014-09-29), pages 53-65, XP029106172, ISSN: 0882-4010, DOI: 10.1016/J.MICPATH.2014.09.014 figure 2 abstract	1-39
X,P	FUREVIK ANETTE ET AL: "New vaccination strategies are required for effective control of winter ulcer disease caused by emerging variant strains of Moritella viscosa in Atlantic salmon", FISH & SHELLFISH IMMUNOLOGY, ACADEMIC PRESS, LONDON, GB, vol. 137, 2 May 2023 (2023-05-02), XP087319543, ISSN: 1050-4648, DOI: 10.1016/J.FSI.2023.108784 [retrieved on 2023-05-02] the whole document	1-39
X	HEIDARSDÓTTIR K.J. ET AL: "Antigen profiles of the fish pathogen Moritella viscosa and protection in fish", JOURNAL OF APPLIED MICROBIOLOGY, vol. 104, no. 4, 1 April 2008 (2008-04-01) , pages 944-951, XP093077927, GB ISSN: 1364-5072, DOI: 10.1111/j.1365-2672.2007.03639.x the whole document	1-39
X	BJÖRNSSON H. ET AL: "Isolation and characterization of an antigen from the fish pathogen Moritella viscosa", JOURNAL OF APPLIED MICROBIOLOGY, vol. 111, no. 1, 1 July 2011 (2011-07-01), pages 17-25, XP093077934, GB ISSN: 1364-5072, DOI: 10.1111/j.1365-2672.2011.05023.x the whole document	1-39

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/066908

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GREGER E ET AL: "Vaccine development for winter ulcer disease, Vibrio viscosus, in Atlantic salmon, Salmo salar L", JOURNAL OF FISH DISEASES, OXFORD, GB, vol. 22, no. 3, 1 May 1999 (1999-05-01), pages 193-199, XP002701223, ISSN: 0140-7775, DOI: 10.1046/J.1365-2761.1999.00163.X [retrieved on 2001-12-24] page 196, column 1, lines 10-14 table 2 "vaccination and challenge experiments"; page 197	1-39
X	KARLSEN CHRISTIAN ET AL: "Atlantic salmon winter-ulcer disease: Combining mortality and skin ulcer development as clinical efficacy criteria againstMoritella viscosainfection", AQUACULTURE, ELSEVIER, AMSTERDAM, NL, vol. 473, 10 February 2017 (2017-02-10), pages 538-544, XP085048916, ISSN: 0044-8486, DOI: 10.1016/J.AQUACULTURE.2017.01.035 the whole document	1-39

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/066908

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments: