

NOVEL FISH PATHOGENIC VIRUS

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FIELD OF THE INVENTION

The present invention pertains to a novel fish pathogenic virus causing disease in fish,
10 to cell cultures comprising said virus, DNA fragments and corresponding proteins of the
said virus, to vaccines on the basis of said virus, DNA and/or protein and to antibodies
reactive with said virus and diagnostic test kits for the detection of said virus.

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GENERAL BACKGROUND

Over the last decades, world-wide a strong increase is seen in the consumption of fish.
This equally regards the consumption of cold water fish such as salmon, turbot, halibut
20 and cod, and tropical fish such as Asian sea bass (barramundi), tilapia, milkfish,
yellowtail, amberjack, grouper and cobia. As a consequence, an increase is seen in the
number and the size of fish farms, in order to meet the increasing needs of the market.
As is known from e.g. animal husbandry, large numbers of animals living closely
together are vulnerable to all kinds of diseases, even diseases hardly known or seen or
25 even unknown before the days of large-scale commercial farming. This is equally the
case in fish farming.

In 2015, outbreaks of a new disease in Asian sea bass (*Lates calcarifer*) were reported
in fish farms in *i.a.* Vietnam and Singapore. The fish were observed to experience Scale
30 Drop Disease-like symptoms. Scale Drop Disease is caused by a virus of the family
Iridoviridae that was recently isolated and described in WO2014/191445. However,
these cases tested negative for Scale Drop Disease using Scale Drop Disease Virus
DNA specific PCR primers. The main clinical signs (not all of these symptoms are
necessarily seen in each and every diseased fish) often associated with this new
35 disease when spotted in the field (e.g. in a fish farm, thus not in a controlled laboratory
environment) can be described as follows.

There is an acute onset of clinical signs with high percentages of morbidity, with many fish affected and up to 60% mortalities (typically 30-70%) within 4-7 days from onset of clinical signs. These clinical signs can be described as follows: Affected fish have
5 generalized skin lesions, become lethargic, and show pronounced inappetence. As the disease progresses, the skin lesions become more severe, leading to a darkening of the skin with pale whitish patches and erosions of the fins and tail giving a ghost-like appearance to the fish. Eyes become swollen and slightly cloudy. The internal signs of the disease often noticed are enlarged spleen and kidneys. Kidneys become friable and
10 easily detachable. Some pallor of the liver can often be observed. The gills become pale as the disease progresses.

15 OBJECT OF THE INVENTION

It is an objective of the present invention to provide the causative agent of this disease. This enables providing detection methods and vaccines aiming at combating the disease.
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SUMMARY OF THE INVENTION

25 It has been found that the causative agent of the disease as described here above is a herpesvirus and a member of the family of Alloherpesviridae. It is an icosahedral virus belonging to the double-stranded DNA-viruses. The virus bears a certain albeit low level of resemblance to viruses of the family Alloherpesviridae, a family of herpesviruses that is pathogenic for fish or amphibians. Viruses of the Alloherpesviridae are enveloped,
30 with icosahedral and spherical to pleomorphic geometries. Their diameter is around 150-200 nm. Genomes are linear and non-segmented, between about 100-250 kbp in length.

The virus of the present invention has a genome of about 130 kbp. Figure 1 shows a
35 phylogenetic tree indicating the relation between the known Alloherpesviridae and the newly found virus. The new virus is referred to in this tree as Lates Calcarifer Herpes

- Virus (LCHV). This tree was made using the program MEGA, version 5, and using standard settings. (MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Koichiro Tamura, Daniel Peterson, Nicholas Peterson, Glen Stecher, Masatoshi Nei and Sudhir Kumar. Mol. Biol. Evol. 28(10): 2731–2739. 2011 doi:10.1093/molbev/msr121 Advance Access publication May 4, 2011).

- The Alloherpesviridae, more specifically the Alloherpesviridae found in fish are summarised in a review paper by Hanson, L. et al., (Viruses 3: 2160-2191, 2011).
- The herpesvirus according to the invention was found in Asian sea bass (*Lateolabrax* *calcarifer*). So far no herpesviruses have been described that are pathogenic to Asian sea bass. It cannot be excluded that the virus is pathogenic for other (sub)-tropical fish as well. Of the known Alloherpesviridae, the Ictalurid herpesvirus 1 (IHV1) is the closest known family member. This virus is pathogenic for channel catfish. However, the overall sequence identity between the virus according to the invention and the Ictalurid herpesvirus 1 at the nucleotide level is well below 60%. The sequence identity with the more distantly related Anguillid Herpesvirus (AHV) and Koi Herpesvirus (KHV) is lower. Table 1 shows a comparison between several of the genes identified in the new herpesvirus and the homologous genes in IHV1. The table shows the most important ORF's identified (left hand column), with the proviso that identified ORF's having less than 300 base pairs are not taken up in the sequential numbering (but simply ignored for the purposes of this table), the position on the DNA (for one representative example of the virus, deposited at Institut Pasteur, see below), the corresponding ORFs in IHV1, and the level of sequence identity at the amino acid level with these known ORFs has been indicated in the table.

- A representative example of the virus has been deposited with the Collection Nationale de Cultures de Microorganismes (CNCM), Institut Pasteur, 25 Rue du Docteur Roux, F-75724 Paris Cedex 15, France, under accession number **CNCM I-5118** (*Lateolabrax* *calcarifer* Herpes Virus).

Table 1 Comparison between novel herpes virus and IHV1

ORF	Frame	Start	stop	Length	AA length	IHV-1 ORF	IHV Id.	Homologue
124	-3	68702	66114	2589	862	1	28%	
148	-2	80856	79603	1254	391	14/15/16	31%	Protein Kinase
149	-3	82211	80982	1230	391	14/15/16	27%	Protein Kinase
150	-1	84211	82781	1431	391	14/15/16	29%	Protein Kinase
174	-3	98246	94749	3498	1403	22	23%	
178	-1	99202	98297	906	303	23	21%	
179	3	99195	100175	981	333	24	36%	
183	1	100990	102237	1248	498	25	56%	DNA helicase
186	1	102601	103485	885	288	27	43%	Putative capsid subunit
189	-2	105687	103690	1998	590	28	38%	Capsid protease
195	3	106164	106808	645	208	30	50%	
198	1	107680	108729	1050	291	33	40%	
201	2	108500	109921	1422	441	34	52%	
202	3	109788	110486	699	238	35	27%	
204	-2	112971	111013	1959	670	37	46%	Allo 7
206	3	113364	116795	3432	1123	39	49%	Major capsid protein
212	-3	118349	117459	891	300	41	34%	
217	-2	121641	118966	2676	891	43	42%	DNA polymerase subunit
220	1	121591	122622	1032	335	44	47%	
224	-2	127533	123625	3909	1355	46	39%	Membrane protein
228	2	127478	128701	1224	371	47	32%	Peptidase
1	-2	531			188	49	45%	dUTPase
136	1	72364	73848	1485	228	5	33%	Deoxyguanosine kinase
6	2	1655	2266	612	318	52	33%	
8	-3	3251	2238	1014	308	53	37%	
10	1	3178	5049	1872	607	54	39%	Allo54
12	1	5098	6288	1191	388	55	30%	
15	-2	11313	7705	3609	1179	56	48%	
21	2	11429	14407	2979	1512	57	58%	DNA polymerase
26	1	14494	16176	1683	1512	57	48%	DNA polymerase
28	-3	17363	16317	1047	345	59	30%	Major envelop protein
32	1	17470	18624	1155	393	60	39%	Allo60
35	-2	19569	18592	978	319	61	33%	
37	2	19505	20488	984	734	62	63%	Terminase
68	1	36622	38133	1512	734	62	51%	DNA packaging terminase
40	3	20418	22394	1977	662	63	39%	Putative helicase
42	1	22276	23874	1599	514	64	42%	Allo64

Legend of Table 1

- ORF: open reading frame (number) in LCHV
 Frame: reading frame on the genome (1,2,3, negative numbers refer to opposite strand)
 Length: length of LCHV ORF in base pairs
 AA length: length of the amino acid hit in BLAST search
 IHV Id.: percentage AMINOACID identity between LCHV ORF and corresponding IHV ORF
 IHV-1: Ictalurid Herpes Virus - 1
 Homologue: Putative function of the protein encoded by the LHCV ORF based on homology with known proteins

15 **DEFINITIONS**

The *wild type form* of the current virus is the virus in a replication competent form, as can be isolated from diseased fish, in particular Asian sea bass, and capable of inducing the same disease in healthy fish of the species of fish from which the virus in its wild type form was isolated. A wild type virus after being inactivated or attenuated, per definition, is capable of inducing disease in its wild type form.

An *isolated virus* is a virus set free from tissue with which the virus is commonly associated in a diseased host in nature and transferred free from other viruses and bacteria to a vessel such as a dish, flask or bio-reactor. An example of an isolated virus is a virus present in a cell culture of a specific cell line in a bioreactor.

An *isolated DNA fragment* is a DNA fragment that is taken out of its natural (whole) DNA as present in the corresponding, natural occurring replication competent organism. Such DNA fragment may be present as such in a stabilising fluid, or may be recombinantly transferred into DNA of another organism. In each case, the DNA fragment is still isolated in the sense of the present invention.

An *isolated protein* is a protein that is taken out of its natural environment, i.e. out of its natural association with the corresponding natural occurring replication competent organism.

A *vaccine* is a pharmaceutical composition that is safe to administer to a subject animal, and is able to induce protective immunity in that animal against a pathogenic micro-organism, i.e. to induce a successful prophylactic treatment. A successful prophylactic

treatment in this sense is a treatment aiding in preventing or ameliorating an infection with that pathogen or a disorder arising from that infection, resulting from a post treatment challenge with the pathogenic pathogen, in particular to reduce its load in the host after such challenge and optionally to aid in preventing or ameliorating one or more clinical manifestations resulting from the post treatment infection with the pathogen.

An *open reading frame* (ORF) is the part of a reading frame that has the potential to code for a protein or peptide. An ORF is a continuous stretch of codons that does not contain a stop codon.

EMBODIMENTS

In a first embodiment the novel virus is characterised as an isolated herpesvirus which is a member of the family of Alloherpesviridae, and which in its wild-type form causes a disease in Asian sea bass which is characterised by the following signs (after intraperitoneal challenge with the virus in a laboratory environment): onset of clinical signs around 3 days after challenge, generalized skin lesions (in a significant amount, typically over 50%, of the cases) leading to darkened skin with pale patches and fin erosions, lethargy with observed loss of swimming equilibrium ("observed" meaning that it is seen but not in all cases, only occurring with extremely lethargic fish), (almost complete) inappetence, increase in opercular respiration rate, and occurrence of mortalities about 2 weeks after challenge.

In a further embodiment the virus has DNA that corresponds to DNA having a sequence according to SEQ ID NO:1. In practice this means that the level of identity over the full length of this DNA is over 70%, preferably over 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, over 99% or even up to 100%. It is noted that it is currently believed that around position 80,000 (out of the 130k base pairs) the virus has an internal repeat. Based on homology with other herpesviruses, it is thus likely that the virus can be present in four genome isomers (as explained by Mahiet et al. in: *Structural variability of the Herpes Simplex Virus 1 genome In Vitro and In Vivo*; Journal of Virology, august 2012, Volume 86, Number 16, pp 8592 – 8601). It may be that the novel virus has one or more additional internal repeats besides the repeat around bp 80,000. However, this has not been established

yet. SEQ ID NO:1 corresponds to one of the possible genome isomers of the novel virus. In yet a further embodiment of the virus, in its DNA, at least 95% of the open reading frames (in particular the ORF's comprising at least 300 basepairs), for example at least 96%, 97%, 98%, 99% or 100%, have at least 80% sequence identity, for
5 example at least 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or even up to 100% identity, with the corresponding open reading frames of the DNA having a sequence according to SEQ ID NO:1.

It is noted that for the purpose of defining the current invention a suitable program for
10 the determination of a level of identity is the nucleotide blast program (blastn) of NCBI's Basic Local Alignment Search Tool, using the "Align two or more sequences" option and standard settings (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

In another embodiment the isolated herpesvirus, which is a member of the family of
15 Alloherpesviridae, has at least one of the identifying characteristics of the virus deposited under Accession Number **CNCM I-5118** at the Collection Nationale de Cultures de Microorganismes (CNCM), Institut Pasteur, Paris, France. This means that the virus can be identified as the novel alloherpesvirus according to the present invention, *i.e.* Lates Calcarifer Herpes Virus. In a further embodiment the identifying
20 characteristics are chosen from the group consisting of 1) the virus having a DNA sequence that over its full length is at least 70% (or at least 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or 100%) identical to the sequence according to SEQ ID NO:1, with the above mentioned proviso regarding the internal repeat; or 2) the virus in its wild type form causes disease
25 in Asian sea bass which is characterised by the following signs: onset of clinical signs around 3 days after challenge, generalized skin lesions (in a significant amount, typically over 50%, of the cases) leading to darkened skin with pale patches and fin erosions, lethargy with observed loss of swimming equilibrium ("observed" meaning that it is seen but not in all cases, only occurring with extremely lethargic fish), (almost complete)
30 inappetence, increase in opercular respiration rate, and occurrence of mortalities occur about 2 weeks after challenge, or 3) the virus comprising a Major Envelope Protein (MEP) gene having a level of identity of at least 80% to the nucleotide sequence as depicted in SEQ ID NO: 2; or 4) the virus comprising a dUTPase gene having a level of identity of at least 80% to the nucleotide sequence as depicted in SEQ ID NO: 4; or the
35 virus comprising a Terminase gene having a level of identity of at least 80% to the nucleotide sequence as depicted in SEQ ID NO: 6; or the virus comprising a

Polymerase gene having a level of identity of at least 80% to the nucleotide sequence as depicted in SEQ ID NO: 8.

In yet another embodiment of the novel herpes virus which is a member of the family of Alloherpesviridae, and which in its wild-type form causes a disease in Asian sea bass, the virus is characterised in that the virus has DNA that corresponds to DNA having a sequence according to SEQ ID NO:1. This means that the virus has DNA that over its full length is at least 70% identical to the DNA according to SEQ ID No.1 (with the above proviso regarding the internal repeat). The level of identity may be higher, for example 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or even 100%.

In still another embodiment of the novel herpesvirus which is a member of the family of Alloherpesviridae, and which in its wild-type form causes a disease in Asian sea bass, the virus is characterised in having DNA wherein at least 95% of the open reading frames (in particular the ORF's comprising at least 300 base pairs), for example at least 96%, 97%, 98%, 99% or 100%, have at least 80% sequence identity, for example at least 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or even up to 100% identity, with the corresponding open reading frames of the DNA having a sequence according to SEQ ID NO:1.

In other embodiments, the novel virus can be distinguished from known members of the Alloherpesviridae on the basis of the coding DNA sequences of its Major Envelope Protein (ORF28) and its dUTPase (ORF1). It turned out that the Major Envelope Protein of the virus has a level of sequence identity with the MEP of even the nearest of the other species of the Alloherpesviridae of only 30%. The dUTPase has a level of sequence identity of only 45% with the nearest dUTPase of the other species of the Alloherpesviridae. Typical examples of the DNA sequence encoding the MEP and dUTPase are shown in SEQ ID NO: 2 and 4 respectively. Their respective amino acid sequences, i.e. the proteins encoded by the DNA fragments according to SEQ ID NO:2 and NO:4 (the terms "encoded by" not excluding that other DNA leads to the same protein, or in other words: "encoded by a specific DNA sequence" means that the protein can be synthesized based on the specific sequence, but possibly also by using another sequence), are shown in SEQ ID NO: 3 and 5. It will be understood that, for the particular proteins embraced herein, natural variations can exist between individual representatives of the causative agent. Genetic variations leading to minor changes in

e.g. the Major Envelope Protein sequence do exist. This is equally true for the dUTPase. First of all, there is the so-called "wobble in the second and third base" explaining that nucleotide changes may occur that remain unnoticed in the amino acid sequence they encode: e.g. triplets TTA, TTG, TCA, TCT, TCG and TCC all encode

5 Leucine. In addition, minor variations between representatives of the new virus according to the invention may be seen in amino acid sequence. These variations can be reflected by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence. Amino acid substitutions which do not essentially alter biological and immunological

10 activities, have been described, e.g. by Neurath et al in "The Proteins" Academic Press New York (1979). Amino acid replacements between related amino acids or replacements which have occurred frequently in evolution are, inter alia, Ser/Ala, Ser/Gly, Asp/Gly, Asp/Asn, Ile/Val (see Dayhof, M.D., Atlas of protein sequence and structure, Nat. Biomed. Res. Found., Washington D.C., 1978, vol. 5, suppl. 3). Other

15 amino acid substitutions include Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Thr/Phe, Ala/Pro, Lys/Arg, Leu/Ile, Leu/Val and Ala/Glu. Based on this information, Lipman and Pearson developed a method for rapid and sensitive protein comparison (Science 227, 1435-1441, 1985) and determining the functional similarity between

20 homologous proteins. Such amino acid substitutions of the exemplary embodiments of this invention, as well as variations having deletions and/or insertions are within the scope of the invention. This explains why e.g. MEP and dUTPase, when isolated from different representatives of the virus according to the invention may have homology levels that are significantly below 100%, while still representing the MEP or dUTPase of the virus according to the invention. Typically, a protein that is either a MEP or dUTPase

25 according to the invention has a sequence identity of at least 70% to the amino acid sequences of SEQ ID NO:3 and SEQ ID NO:5 respectively, thus having a sequence identity of 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or even 100% with these sequences.

30 The embodiments (A-G) relating to these proteins (i.e. MEP and dUTPase) in particular thus are as follows:

A: an isolated herpesvirus comprising a Major Envelope Protein (MEP) gene, characterized in that the virus is a member of the family Alloherpesviridae, the virus

35 causes disease in Asian sea bass, and the nucleotide sequence of the MEP gene has a level of identity of at least 80% (for example 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91,

92, 93, 94, 95, 96, 97, 98, 99% or 100%) to the nucleotide sequence as depicted in SEQ ID NO: 2.

5 B: an isolated herpesvirus comprising a dUTPase gene, characterized in that the virus is a member of the family Alloherpesviridae, the virus causes disease in Asian sea bass, and the nucleotide sequence of the dUTPase gene has a level of identity of at least 80% (for example 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or 100%) to the nucleotide sequence as depicted in SEQ ID NO: 4.

10 C: an isolated herpesvirus having both the MEP gene and the dUTPase gene, characterized in that the nucleotide sequence of the MEP gene has a level of identity of at least 80% to the nucleotide sequence as depicted in SEQ ID NO: 2 and the nucleotide sequence of the dUTPase gene has a level of identity of at least 80% (for example 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or 15 100%) to the nucleotide sequence as depicted in SEQ ID NO: 4.

D: an isolated herpesvirus comprising a Major Envelope Protein (MEP) gene, characterized in that the virus is a member of the family Alloherpesviridae, the virus causes disease in Asian sea bass, and the MEP gene reacts in a PCR reaction with a primer set as depicted in SEQ ID NO: 21 and 22 to give a PCR product of 277 +/- 10 base pairs.

E: an isolated herpesvirus comprising a dUTPase gene, characterized in that the virus is a member of the family Alloherpesviridae, the virus causes disease in Asian sea bass, and the dUTPase gene reacts in a PCR reaction with a primer set as depicted in SEQ ID NO: 23 and 24 to give a PCR product 346 +/- 10 base pairs.

F: an isolated herpesvirus comprising the MEP gene and the dUTPase gene, characterized in that the MEP gene reacts in a PCR reaction with a primer set as depicted in SEQ ID NO: 21 and 22 to give a PCR product of 277 +/- 10 base pairs and the dUTPase gene reacts in a PCR reaction with a primer set as depicted in SEQ ID NO: 23 and 24 to give a PCR product of 346 +/- 10 base pairs.

G: an isolated herpesvirus, characterized in that the nucleotide sequence of the MEP gene has a level of identity of at least 80% (or at least 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or 100%) to the nucleotide sequence as depicted

in SEQ ID NO: 2 and the nucleotide sequence of the dUTPase gene has a level of identity of at least 80% (or at least 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or 100%) to the nucleotide sequence as depicted in SEQ ID NO: 4 and in that the viral DNA reacts in a PCR reaction with a primer set as depicted in SEQ ID NO: 21 and 22 to give a PCR product of 277 +/- 10 base pairs and reacts in a PCR reaction with a primer set as depicted in SEQ ID NO: 23 and 24 to give a PCR product of 346 +/- 10 base pairs.

The embodiments D through G make use of a PCR-test using primer sets for the Major Envelope Protein gene sequence or the dUTPase gene sequence of a virus according to the invention. Two different primer sets of which the sequence is depicted in SEQ ID NO: 21-22 and SEQ ID NO: 23-24 were elected. The PCR-test using the first primer set (SEQ ID NO: 21-22) that reacts with the Major Envelope Protein gene of the virus uses the two primers LCHV MEP FW and LCHV MEP REV (see table 2b in the examples section). The PCR-test using the second primer set (SEQ ID NO: 23-24) reacts with the dUTPase gene of the virus and uses the two primers LCHV dUTP FW and LCHV dUTP REV (see Table 2c in the examples section). The tests, which are described in more detail in the Examples section, are standard PCR tests. If analysis of the PCR-product of the first primer set reveals a PCR product of approximately 277 base pairs or if analysis of the PCR-product of the second primer set reveals a PCR product of approximately 346 base pairs, and the virus is a member of the Alloherpesviridae and causes disease in Asian sea bass, this unequivocally demonstrates that the analysed virus is a virus according to the invention. For the purpose of the present invention, a PCR product of approximately 277 base pairs is a PCR product with a length of between 277 + 10 and 277 - 10 base pairs. A PCR product of approximately 346 base pairs is a PCR product with a length of between 346 + 10 and 346 - 10 base pairs.

Other embodiments (H-K) relating to other proteins specific for the novel virus, *i.e.* a Terminase and a Polymerase, in particular are as follows:

H: an isolated herpesvirus comprising a Terminase gene, characterized in that the virus is a member of the family Alloherpesviridae, the virus causes disease in Asian sea bass, and the Terminase gene reacts in a PCR reaction with a primer set as depicted in SEQ ID NO: 25 and 26 to give a PCR product of 585 +/- 10 base pairs.

I: an isolated herpesvirus comprising a Polymerase gene, characterized in that the virus is a member of the family Alloherpesviridae, the virus causes disease in Asian sea bass, and the Polymerase gene reacts in a PCR reaction with a primer set as depicted in SEQ ID NO: 27 and 28 to give a PCR product 314+/- 10 base pairs.

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J: an isolated herpesvirus comprising the Terminase gene and the Polymerase gene, characterized in that the Terminase gene reacts in a PCR reaction with a primer set as depicted in SEQ ID NO: 25 and 26 to give a PCR product of 585+/- 10 base pairs and the Polymerase gene reacts in a PCR reaction with a primer set as depicted in SEQ ID NO: 27 and 28 to give a PCR product of 314 +/- 10 base pairs.

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K: an isolated herpesvirus, characterized in that the nucleotide sequence of the Terminase gene has a level of identity of at least 80% (or at least 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or 100%) to the nucleotide sequence as depicted in SEQ ID NO: 6 and the nucleotide sequence of the polymerase gene has a level of identity of at least 80% (or at least 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or 100%) to the nucleotide sequence as depicted in SEQ ID NO: 8 and in that the viral DNA reacts in a PCR reaction with a primer set as depicted in SEQ ID NO: 25 and 26 to give a PCR product of 585 +/- 10 base pairs and reacts in a PCR reaction with a primer set as depicted in SEQ ID NO: 27 and 28 to give a PCR product of 314 +/- 10 base pairs.

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The embodiments H through K make use of a PCR-test using primer sets for the Terminase gene sequence or the Polymerase gene sequence of a virus according to the invention. Two different primer sets of which the sequence is depicted in SEQ ID NO: 25-26 and SEQ ID NO: 27-28 were elected. The PCR-test using the first primer set (SEQ ID NO: 25-26) that reacts with the Terminase gene of the virus uses the two primers LCHV TER FW and LCHV TER REV (see table 2d in the examples section). The PCR-test using the second primer set (SEQ ID NO: 27-28) reacts with the Polymerase gene of the virus and uses the two primers LCHV POL FW and LCHV POL REV (see Table 2e in the examples section). The tests, which are described in more detail in the Examples section, are standard PCR tests. If analysis of the PCR-product of the first primer set reveals a PCR product of approximately 585 base pairs or if analysis of the PCR-product of the second primer set reveals a PCR product of approximately 314 base pairs, and the virus is a member of the Alloherpesviridae and causes disease in Asian sea bass, this unequivocally demonstrates that the analysed

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virus is a virus according to the invention.

On the basis of the above described DNA coding sequences for the Major Envelope Protein and the dUTPase of the novel virus, also the following embodiments L-O of the present invention are provided:

L: a (isolated) DNA fragment comprising a gene encoding a Major Envelope Protein characterized in that said gene has a level of identity of at least 80% (or at least 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or 100%) to the nucleotide sequence of the MEP gene as depicted in SEQ ID NO: 2, and M: a (isolated) Major Envelope Protein encoded by this DNA fragment.

N: a (isolated) DNA fragment comprising a gene encoding an dUTPase characterized in that said gene has a level of identity of at least 80% (or at least 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or 100%) to the nucleotide sequence of the dUTPase gene as depicted in SEQ ID NO: 4, and O: a (isolated) dUTPase encoded by this DNA fragment.

It is currently believed that several other genes could also be useful in identifying the novel virus according to the invention. One of these genes is the gene corresponding to ORF224 (SEQ ID NO:10), which encodes for a membrane (glyco)protein (SEQ ID NO:11). Yet another gene is the gene homologous to the Ictalurid herpes virus 1 (IHV1) TK (Thymidine kinase) gene. In IHV-1 this gene corresponds to ORF5 (Hanson et al., Virology. 1994 Aug 1; 202(2):659-64). The novel virus has a conserved domain "Deoxyribonucleoside kinase" with an identity of 33% at the amino acid level with ORF 5 of IHV-1. Another gene (SEQ ID NO:12) for identifying the novel virus is the gene comprising ORF206, coding for a Major Capsid Protein (SEQ ID NO:13).

Still another embodiment according to the present invention pertains to any (isolated) DNA fragment having an open reading frame having a length of at least 100 nucleotides, wherein the DNA has at least 80% (or at least 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or 100%) sequence identity with an open reading frame of the DNA that has a sequence according to SEQ ID NO:1. Although a length of 30-40 nucleotides has found to be sufficient to distinguish the DNA of a virus according to the invention over the DNA of any virus publicly known, a practical relevant length, in particular for corresponding sub-unit vaccines based on the corresponding

protein, is at least 100 nucleotides (or at least 150, 200, 250 or even at least 300 nucleotides) so as to correspond to a protein having a relevant and distinguishing 3D conformity that corresponds to relevant immunogenic epitopes of a viral protein. In a further embodiment therefor, the invention also pertains to a (isolated) protein encoded
5 by such a DNA fragment.

In an embodiment, the invention also pertains to a cell culture (i.e an artificial culture of a restricted number of types of cells, also called a cell line, in an artificial culture vessel; also described a process by which cells are grown under controlled conditions outside
10 of their natural environment) comprising the novel virus according to the invention in a replication competent form. Several fish cell lines are potentially useful to support the replication of a virus according to the invention. An example of a cell line that can be used to grow the virus according to the invention is a cell line from brain cells of Asian sea bass. Methods for the isolation of such a cell line have been described i.a. by
15 Hasoon et al., in In Vitro Cell. Dev. Biol. – Animal 47: 16-25 (2011). Another example of a cell line that is potentially useful to support replication of the virus is disclosed by Chi et al: "Persistent infection of betanodavirus in a novel cell line derived from the brain tissue of barramundi *Lates calcarifer*", Chi SC, Wu YC, Cheng TM, Dis Aquat Organ. 2005 Jun; 65(2):91-8. PMID: 16060261. It has also been established that a primary cell
20 culture from Seabass fins using common methods is useful for supporting replication of the virus. Other cell lines that may be useful for growing the virus are cells from the skin, brain, heart, *i.e.* organs wherein the virus likely replicates.

In again another embodiment, the invention pertains to a vaccine for combating
25 herpesvirus disease in fish, wherein said vaccine comprises a herpesvirus according to the invention, or an immunogenic protein as described here above and a pharmaceutically acceptable carrier. Such a carrier may be as simple as water, as long as it is suitable for administration of the material in clinically relevant amounts without causing unacceptable side effects. Typical carriers are emulsions of oil and water,
30 suspensions of insoluble adjuvants (typically aluminium or other salts, or large immune stimulating polymeric molecules) in water or solutions of soluble adjuvants such as saponins, PAMP's, carbopol or other immune stimulating molecules. Typically the carrier comprises stabilisers and preservatives as is commonly known in the art. In a further embodiment the vaccine comprises a herpesvirus according to the invention in a
35 live attenuated (*i.e.* replication competent but no longer capable of inducing the full suite of symptoms as induced by the originating wild type pathogen) or inactivated form.

- Experimental vaccines for herpes simplex virus have been designed with different technologies. Vaccines consisted of peptides, (recombinant) viral proteins, mixtures of viral proteins, whole and fractionated killed virus (see for example Yasumoto, S. et al. in Fish Pathology 41: 141-145 (2006) which describes a vaccine comprising a inactivated whole koi herpes virus, trapped within a liposomal compartment), replication-defective viruses, and attenuated replication-competent viruses (as summarized by Koelle, D.M. and L. Corey. 2003: Recent Progress in Herpes Simplex Virus Immunobiology and Vaccine Research. Clin Microbiol Rev. 16(1): 96–113). Each of the approaches has specific advantages and disadvantages, which have been discussed by Stanberry in 2000 (Stanberry, L. R., A. L. Cunningham, A. Mindel, L. L. Scott, S. L. Spruance, F. Y. Aoki, and C. J. Lacey. 2000: Prospects for control of herpes simplex virus disease through immunization. Clin. Infect. Dis. 30:549-566.)
- It is known that *in vitro* serial passage of virulent herpes virus strains in cell culture results in the generation of attenuated progeny, or in other words non-virulent replication-competent strains that evoke a protective immune response without causing clinical symptoms of disease. Attenuation was for example achieved for the Marek's disease virus (MDV), with a protective vaccine known as Rispens or CVI988 by *in vitro* serial passage of a virulent virus until the resulting isolate became avirulent (Rispens BH, Vloten H, Mastenbroek N, Maas HJ, Schat KA. 1972: Control of Marek's disease in the Netherlands. 1. Isolation of an avirulent Marek's disease virus (strain CVI988) and its use in laboratory vaccination trials. Avian Dis. 16:108–125). Correspondingly, it is described that multiple genes, often within pathways involving DNA replication and transcriptional regulation, are involved in *de novo* attenuation of MDV and provide targets for the rational design of future MD and thus corresponding herpes virus vaccines. Attenuation by serial passaging has also been described for fish herpes viruses (see i.a. Noga, E.J. et al., Can. J. Fish. Aquat. Sci. 38: 925-929, 1981).
- As is commonly known, attenuation of viruses can be spontaneous or can be induced by drugs (mutagenic or other nature such as for example UV light; see e.g. *Mutation Research* 768, 2016, 53-67 and *J. gen. Virol*, 1985, **66**, 2271-2277).
- The underlying genetic mechanisms behind attenuation often remained poorly understood, but genetic changes (mutations, deletions, etc.) and/or the accumulation thereof in the viral genome are the basis for attenuation of herpes virus strains.

Mutations may relate to multiple viral mechanisms including replication capacity, spread of the virus, etcetera. Certain molecular pathways essential for virus replication and infectivity *in vivo* may be not essential for replication culture, and those genes involved in such pathways are likely more prone to genetic alterations during long-term culture.

5

If such random mutations and deletions in the genome are characterized, and with the development of next generation sequencing techniques it has become relatively simple to sequence the entire genome of large viruses such as herpesviruses, this enables rational design of attenuation. From literature on herpes virus attenuation, multiple genes have come forward as possible targets where gene dysfunction leads to functional attenuation of the virus. A detailed overview of genes involved in virus replication that could have undergone mutations in a live attenuated herpes simplex vaccine is given by Roizman and Knipe in 2001 (Roizman, B., and D. M. Knipe: Herpes simplex viruses and their replication, p. 2399-2459. In D. M. Knipe, P. M. Howley, and D. E. Griffin (ed.), Fields virology, 4th ed., vol. 2. Lippincott, Philadelphia, Pa).

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15

Furthermore, such mutations can be used to setup a vaccine approach with a discontinuously replicating virus. The mutated virus is grown in a genetically engineered cell line that provides the required non-mutated gene in trans. For example, when herpes simplex virus with a deletion of UL22, the late gene encoding gH, infects a non-complementing cell, the progeny virions can exit the cell but cannot infect a secondary cell (Koelle and Corey, 2003 as cited here above).

20

Below is a list of herpes virus genes of which dysfunction or deletion has been described to lead to functional attenuation of Koi Herpes Virus, an alloherpesvirus, or other herpesviruses. These genes are target genes for attenuation of the herpes virus according to the present invention:

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1) The thymidine kinase (TK) gene of Koi Herpes Virus. A TK gene has also been described in Channel catfish herpesvirus (CCV), a virus relatively closely related to the virus described in the present invention (Hanson LA, Kousoulas KG, and RL Thune. 1994: Channel catfish herpesvirus (CCV) encodes a functional thymidine kinase gene: elucidation of a point mutation that confers resistance to Ara-T. Virology 202(2):659-64).

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2) The d-UTPase gene of Koi Herpes Virus.

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3) The gene encoding ORF57 of Koi Herpes Virus, as given in Genbank accession N° NC_009127 where the ORF57 start and stop codon are located at position 99382 and 100803 (Boutier M, Ronsmans M, Ouyang P, Fournier G, Reschner A, et al. (2015): Rational Development of an Attenuated Recombinant Cyprinid Herpesvirus 3 Vaccine
5 Using Prokaryotic Mutagenesis and In Vivo Bioluminescent Imaging. PLoS Pathog 11(2): e1004690).

4) The gD (EHV en BHV) / gp50 (PRV) gene as found in bovine herpesvirus, equine herpesvirus en pseudorabiesvirus (Aujeszky's disease). This gene encodes a
10 glycoprotein to which neutralizing antibodies can be generated.

The invention also pertains to a method of prophylactically a treating a an animal (i.e. for treating an animal to prevent a post treatment infection with the corresponding wild type pathogen) with a vaccine as described here above, comprising systemically
15 administering the vaccine to the animal. Systemically administering a vaccine means to administer the vaccine such that it reaches the circulatory system of the body (comprising the cardiovascular and lymphatic system), thus affecting the body as a whole rather than a specific locus such as the gastro-intestinal tract. Systemic administration can be performed e.g. by administering the antigens into muscle tissue
20 (intramuscular), into the dermis (intradermal), underneath the skin (subcutaneous), underneath the mucosa (submucosal), in the veins (intravenous), into the body cavity (intraperitoneal) etc.

The invention is also embodied in an antibody or antiserum reactive with a virus
25 according to the invention and in a diagnostic test kit for the detection of antibodies reactive with a virus according to the invention or with antigenic material thereof, wherein the test kit comprises a virus according to the invention or antigenic material thereof. The invention is also embodied in a diagnostic test kit for the detection of a herpesvirus according to the invention or antigenic material thereof, wherein said test kit
30 comprises antibodies reactive with a virus according to the invention or with antigenic material thereof or a PCR primer set as described here above.

The invention will now be further explained using the following examples.

EXAMPLES

Example 1: Discovery of Lates Calcarifer Herpes Virus**5 Collection of serum and tissue samples for isolation of an infectious agent**

Diseased fish were observed in Asian Seabass (*Lates calcarifer*) fish farm in Singapore. The diseased fish appeared with clinical symptoms which to the farmer appeared similar to Scale drop disease (Gibson-Kueh *et al.*, J Fish Dis. 2012 Jan;35(1):19-27. doi: 10.1111/j.1365-2761.2011.01319.x. PMID: 22103767; de Groof *et al.*, PLoS Pathog. 10 2015 Aug 7;11(8):e1005074. doi: 10.1371/journal.ppat.1005074. PMID: 26252390) However, when disease symptoms were studied more closely it appeared that the diseased fish showed a more acute infection (3-10 days instead of more than 15 days) with higher morbidity. The skin lesions were less severe, but more generalized compared to scale drop disease. The skin lesions differed from scale drop disease 15 lesions in that the whole fish was darker and duller, with patches of pale mucus. Some scale loss was observed, but it was not prominent, nor was it the predominant clinical sign. This is different from scale loss caused by Scale drop disease virus which has localized, patchy lesions that are more deeply affected by necrosis with severe scale loss. Scale drop disease virus also typically causes a more chronic outbreak. Based on 20 the observed difference, and the fact that a Scale drop disease virus PCR gave negative results, presence of a different infectious viral agent was suspected based on clinical observations on the affected farm. It was decided to do a follow up study aimed at virus isolation, and a new virus was found.

25 Other observations on the diseased fish, apart from scale loss, were acute episodes of lethargy, severe inappetence, cloudy/swollen eyes and high mortalities (up to 30-70% of affected fish). Samples (serum, kidneys, spleens) were taken from affected fish. Pooled serum samples were stored at -70°C until further analysis. Pooled kidney samples were kept at +4°C until homogenization the next day.

30

Tissue homogenization of tissue samples for isolation of an infectious agent

Kidney samples were homogenized by manual grinding using homogenizing sticks in SVDB (Standard Vaccine Dilution buffer = PBS) and sample was finally diluted 1:9 (w/v) in SVDB and then pre-treated in gentamicin for 1hr. The homogenized sample was 35 centrifuged at 5,500 rpm at +4°C for 10 minutes, and cell-free supernatant was subsequently collected.

Seabass brain (SBB) cells were seeded at 2×10^4 cells/cm² in EMEM + 10% FBS + gentamycin + amphotericin in T75 flasks on the day before inoculation of the monolayer. For this experiment, cells were grown in medium to which also HEPES and sodium bicarbonate were added, and cells were optimized to grown in these conditions without CO₂. The next day, the medium (15 mL) was exchanged with fresh medium to which 0.1 mL or 0.3 mL of the undiluted cell free supernatant was added. The flasks were incubated at 28°C. CPE was observed in the flasks to which the undiluted cell free supernatant was added (both 0.1 and 0.3 mL) after 3 days and harvested on day 5 (passage 1). The presumed infectious agent was designated V511. The CPE-causing agent was passaged an additional 3 times on SBB cells according to the protocol above. The CPE causing agent was designated V511/SBB_4P.

Virus detection on culture supernatant of CPE-causing agent in cells and serum of affected fish

Serum samples of affected fish and culture supernatant of passage 4 harvest V511/SBB_4P of the CPE-causing agent were analyzed using VIDISCA-454 technology described by De Vries *et al.* (2011) PLoS ONE 6(1):e16118. In both types of samples, sequences were obtained that were suspected to originate from a novel fish pathogen. These sequences were used to derive PCR primers for conventional PCR and quantitative PCR (see Table 2a-e). Blasting of the new sequences revealed that the CPE-causing agent in the culture supernatant and the suspected infectious agent in serum show a certain level of homology to viruses of the family of *Alloherpesviridae*. This new virus is henceforward called Lates Calcarifer Herpes Virus (LCHV).

25

Full Genome sequencing

The virus culture supernatant sample was centrifuged for 10 minutes at 10,000 x g and treated with TurboDNase (Thermofisher) as described (de Vries M, et al. PLoS One. 2011;6(1):e16118. doi:10.1371/journal.pone.0016118), after which nucleic acids were extracted by Boom extraction method (Boom R, et al. J Clin Microbiol. 1990;28(3):495-503). The samples were sheared using dsDNA Fragmentase (New England Biolabs). The sheared samples were purified with AMPure XP beads (agencourt AMPure XP PCR, Beckman Coulter) in a ratio 1:1.8 (sample:beads) to remove the enzymes. After purification the samples were end repaired with DNA polymerase I, Large (Klenow) Fragment (New England Biolabs). The end repaired samples were purified with AMPure XP beads (agencourt AMPure XP PCR, Beckman Coulter) in a ratio 1:1.8

(sample:beads) to remove the enzymes, after which the samples were A-tailed by using Klenow Fragment (3'→5' Exo-) (New England Biolabs). The samples were purified with AMPure XP beads (agencourt AMPure XP PCR, Beckman Coulter) in a ratio 1:1.8 (sample:beads) to remove the polymerases. Bubble adaptors from the NEBNext Multiplex Oligos for Illumina (New England Biolabs) were ligated to the A-tailed samples, by use of T4 ligase (Thermofisher). A size selection was performed by use of AMPure XP beads (agencourt AMPure XP PCR, Beckman Coulter) first in a ratio 1:0.5 (sample:beads) to ensure that most fragments with a size bigger than 400 bp were removed, followed by adding additional AMPure XP beads (agencourt AMPure XP PCR, Beckman Coulter) to the supernatant to get to a final ratio of 1:0.85 (sample:beads) to bind DNA fragments between 200-400 bp and to remove fragments smaller than 200 bp. After the size selection the Bubble adaptors were opened by using USER enzyme from the NEBNext Multiplex Oligos for Illumina (New England Biolabs). Next, a 28 cycle PCR was performed with adaptor specific primers from the NEBNext Multiplex Oligos for Illumina (New England Biolabs) and Q5 hotstart mastermix (New England Biolabs); 30 sec 98°C, and cycles of 10 sec 98°C and 75 sec 65°C, followed by 5 min 65°C. After PCR the samples underwent size selections by use of AMPure XP beads (agencourt AMPure XP PCR, Beckman Coulter) in a ratio 1:0.5 (sample:beads) to remove fragments with a size bigger than 400 bp, and to the supernatant additional AMPure XP beads (agencourt AMPure XP PCR, Beckman Coulter) were added to get to a final ratio of 1:0.85 (sample:beads) to bind DNA fragments between 200-400 bp and to remove fragments smaller than 200 bp. Next the concentration of the DNA was measured via Qubit dsDNA HS Assay Kit (Thermofisher), the size was checked on the bioanalyzer with a High Sensitivity DNA Analysis Kit. The DNA was diluted to a concentration of 2.49 ng/μl. They were sequenced by use of the MiSeq (Illumina) using paired end sequencing and the v2 kit (Illumina).

Phylogenetic analysis

Initial phylogenetic analysis was based on a 208 bp DNA fragment (SEQ ID NO: 14) of the Lates Calcarifer Herpes Virus that was discovered in samples of the disease outbreak. This fragment shows homology at the translated nucleotide level with ORF62 of Ictalurid Herpes virus 1 NP_041153.2 and other fish viruses. Nucleotide and protein sequence alignments were generated using the BLAST Basic local alignment search tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and the multiple sequence alignment tool ClustalW. Phylogenetic trees were created with MEGA5 software using the neighbor-joining method, with partial deletion in case of gaps or insertions (MEGA5: Molecular

Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Koichiro Tamura, Daniel Peterson, Nicholas Peterson, Glen Stecher, Masatoshi Nei and Sudhir Kumar. Mol. Biol. Evol. 2011 28(10): 2731–2739. 2011 doi:10.1093/molbev/msr121. The results of this phylogenetic analysis are presented in Figure 1, depicting the phylogenetic tree of LCHV, based on homology analysis of the 208 bp DNA fragment of LCHV with IHV1 ORF62. The phylogenetic analysis confirmed that LCHV is a newly discovered virus in the family of the *Alloherpesviridae*.

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Example 2: Detection of Lates Calcarifer Herpes Virus using PCR, qPCR analysis

Primer design

PCR primers were designed on a 208 bp DNA fragment of the Lates Calcarifer Herpes Virus that was discovered in samples of the disease outbreak. This fragment shows homology at the translated nucleotide level with ORF62 of Ictalurid Herpes virus 1 NP_041153.2. Primers were also designed on the MEP, dUTPase, Terminase and Polymerase genes.

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Table 2a: Primers designed on 208 bp DNA fragment of Lates Calcarifer Herpes Virus

Primer set	Product	Sequence (5' to 3')	Product length (bp)	Seq ID
Set 1	Forward	GAAATCTATCGGGACCAATGTC	208	Seq ID 15
	Reverse	GAGCCGAATATGCGACTGAGG		Seq ID 16
Set 2	Forward	GGACCAATGTCAAAGTCGTG	94	Seq ID 17
	Reverse	GTCGATCCTAGCAGGAAAGC		Seq ID 18
Set 3	Forward	CGGGACCAATGTCAAAGTCG	141	Seq ID 19
	Reverse	TACACCGCCGTGAAGATTCC		Seq ID 20

25

Table 2b: Primers designed on MEP of Lates Calcarifer Herpes Virus

Primer set	Product	Sequence (5' to 3')	Product length (bp)	Seq ID
LCHV	Forward	ATCACATCGTCACCTACCGC	277	Seq ID 21
MEP	Reverse	ATGGTCTGTCGCTTTCGGAG		Seq ID 22

Table 2c: Primers designed on dUTPase of Lates Calcarifer Herpes Virus

Primer set	Product	Sequence (5' to 3')	Product length (bp)	Seq ID
LCHV dUTPase	Forward	GGCGTTCCCGAAAATCGAAG	346	Seq ID 23
	Reverse	GGGGATAACCACCAAGTTGGG		Seq ID 24

Table 2d: Primers designed on Terminase of Lates Calcarifer Herpes Virus

Primer set	Product	Sequence (5' to 3')	Product length (bp)	Seq ID
LCHV TER	Forward	GTCGTCATCTCTACCGCTCGTCC	585	Seq ID 25
	Reverse	CTTACCGCACTGCCTAGGCGCCAG		Seq ID 26

5

Table 2e: Primers designed on Polymerase of Lates Calcarifer Herpes Virus

Primer set	Product	Sequence (5' to 3')	Product length (bp)	Seq ID
LCHV POL	Forward	GGTACGACTGGTCCCAGATCCAGG	314	Seq ID 27
	Reverse	GACGTGTTTGATCTTGTGCTGAC		Seq ID 28

10

PCR & Gel Electrophoresis

Conventional PCR was performed using a Veriti 96 well thermal cycler (Applied Biosystems). A master mix was made containing 1x Supertaq buffer, 0.02 U/μl Supertaq enzyme, 0.2 mM deoxyribose nucleoside triphosphates (dNTPs), 1 μM forward and 1 μM reversed primer. For each sample 2.0 μl DNA template was added in 48 μl PCR mix, 2 μL sterile water was used as negative control. A PCR program was designed starting with 60 seconds initialization at 95°C, followed by 40 repeats of denaturation, annealing and elongation for 30 seconds each at respectively 95°C, the primer set-specific annealing temperature based on T_m of the primers, and 72°C. The program ended with a final elongation at 72°C for 10 minutes. Samples were loaded with 1x ethidium bromide in 1.5% agarose gel and 1x TAE buffer at 115 Volt for 60 minutes.

15

20

qPCR Analysis

Quantitative Polymerase Chain Reactions were performed using a BioRad CFX 96 system. qPCR experiments were performed using Probe Fast Master Mix together with a sequence-specific probe. Each reaction consisted of 18 μl master mix containing 1x Probe Fast q-PCR Master Mix (KAPA), 200 nM forward primer, 200 nM reversed primer and 200 nM probe. Primer pair 2 was used for qPCR analysis, with an optimized T_m of 60.7°C. The probe DNA sequence was CGCGGGATGACCTCTTCTCG (SEQ ID

25

NO:31) and the labels used were 5' 6FAM and 3' TAMRA. To 18 µl of the master mix, 2 µl DNA template was added. Each reaction was performed in duplicate and all plates were spun 4 minutes at 2,200 x g before insertion in the CFX system. Amplification occurred using a program starting at 95.0°C for 3 minutes, followed by 40 repeats at 95.0°C for 3 seconds and 60.7°C for 30 seconds.

Standard Line

A dilution series containing a pUC57 vector with Lates Calcarifer Herpes Virus-identity-sequence construct (synthesized by GenScript [the 208 bp fragment as mentioned above was synthesized and cloned in a plasmid vector]) was used as positive control, indicator for efficiency and accuracy and for sample quantification. The vector was dissolved and diluted in water in a range of 1.0×10^1 copies / 2µL to 1.0×10^9 copies / 2 µL per qPCR reaction. The dilution series were included in all qPCR experiments and stored at -20°C. Quantification of Lates Calcarifer Herpes Virus DNA in samples was done by the supporting software (CFX-Manager version 3.1), which used the obtained data from the dilution series to create a standard line. All qPCR experiments that were performed showed an efficiency between 98.0% and 100.2% and an accuracy between 0.997 and 0.999 underlining robustness of the qPCR method that was designed for quantification of LCHV DNA.

Example 3: Experimental infection of the novel infectious agent in Asian Seabass

Experimental infection of V511/SBB_4P in Asian Seabass (*Lates calcarifer*)

In this experiment, intra-peritoneal and cohabitation infection of fish with V511 was conducted to investigate if V511 is the cause of the disease outbreaks in the field. Infection was followed by sampling of different organs at various time points to determine the course of infection.

Table 3: Treatment groups and tank allocation.

Tank	Group	Dose (IP)	No. fish
1A	V511/SBB_4P - Neat	0.3 mL	25
1B	Cohabitant	-	25

One group of 25 Asian Seabass was challenged by intra-peritoneal (IP) injection with 0.3 mL of undiluted V511 cell free supernatant (CFS) grown on SBB cell line V511/SBB_4P. The viral titer of the CSF was 3.2×10^6 TCID₅₀/mL (see below for titration protocol). A second group of 25 fish were cohabitated in the same tank separated by netting (Table 3). The netting allowed free movement of water between the two tank halves and allowed close proximity, but not direct contact between fish of the two treatment groups. At the time of the start of the experiment, the fish averaged a weight of 18 grams.

10

Fish were starved for about 24 hours prior to the challenge to ensure emptying of the gastro-intestinal tract to reduce the risk of injury. The fish were anaesthetized by sedation with Aquí-S in accordance with standard procedures prior to challenge. The fish in the IP challenged group were netted, sedated and IP injected midline between the base and tip of the pelvic fin. Following challenge, fish were placed in the assigned tank for recovery and observation. Uninfected cohabitant fish were introduced into the adjacent tank partition separated by netting.

15

Mortality and clinical signs were recorded. At days 4, 7, 11, 14 and 18 days post-challenge, 3 fish in the cohabitant group were randomly sampled and subject to necropsy for harvesting of fish tissue for further testing. Sampling included spleen, heart, brain, serum, liver, intestine, gills, skin and kidney in order to better understand the course of infection of the novel agent after infection via the natural route. At days 4, 7, 11, and 14 post- challenge, 3 fish in the IP infected group were randomly sampled and subject to necropsy for harvesting of kidney tissue for further testing. At day 17, spleen, heart, brain, serum, liver, intestine, gills, skin and kidney were sampled from 3 experimentally infected fish. By day 18 post-challenge, all fish were either sampled, or had succumbed to the infection (see Table 6). Samples collection is summarized in Table 4 and Table 5.

25

30

Table 4: Sampling of fish tissue in experimentally IP infected group.

Days post IP infection	IP infection. Pooled organs (3 fish/pool)
4, 7, 11, 14	Kidneys
17	Kidneys, Spleen, Heart Brain, Serum, Liver, Intestine, Gills, Skin

Table 5: Sampling of fish tissue in cohabitant group.

Days post IP infection	Cohabitant infection Pooled by organs (3 fish/pool)
4, 7, 11, 14, 18	Kidneys, Spleen, Heart, Brain, Serum, Liver, Intestine, Gills, Skin

Table 6: Observed clinical signs in IP and cohabitation challenged treatment groups

Clinical signs	Days post challenge																		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Decreased appetite																			
Skin and fin lesions																			
Lethargy																			
Slight gaping and increase in respiratory rate																			
Inappetence - almost no feed intake																			
Loss of equilibrium																			
Darkened skin																			
Obvious gaping and increased respiratory rate																			
Swollen eyes																			
Mortalities																			
IP group																			
Cohabitation group																			

The results presented in Table 6 show that both IP and cohabitation challenge route were able to produce similar clinical signs to those observed at the index Asian Seabass farm. The gross clinical signs observed were lethargy, inappetence, skin, fin and eye lesions, which were also similarly observed in affected farmed fish at the index farm.

5

This shows that in a controlled laboratory environment (where no other pathogens and less stressors are present when compared to a field environment such as a fish farm) the typical symptoms after (IP) challenge are 1) onset of clinical signs around 3 days after challenge, 2) generalized skin lesions possibly leading to darkened skin with pale patches and fin erosions, 3) loss of swimming equilibrium from extreme lethargy, 4) almost complete inappetence, 5) increase in opercular respiration rate, and 6) occurrence of mortalities occur about 2 weeks after challenge. Swollen and cloudy eyes may be observed in some fish. Mortality and percentage cumulative mortality record is shown in Table 7.

Table 7: Daily mortality and percentage cumulative mortality record*.

	Group	Days post challenge				
		0	1-15	16	17	18
Daily mortality	V511 IP	0	0	1	8	1
	Cohabitant	0	0	0	7	3
% cumulative mortality	V511 IP	0	0	8	69	77
	Cohabitant	0	0	0	54	77

* Does not include fish sampled for tissue collection.

V511/SBB_4P virus supernatant transmitted from IP challenged to naïve cohabitant fish.

Both groups experienced similar clinical symptoms. The clinical symptoms were also similar to those observed at the index farm (initial outbreak). The IP infected group experienced more acute and severe disease compared to the cohabitation group, with disease signs appearing within 3 days post-infection. In comparison, cohabitation challenged fish were showing initial clinical signs on day 7 post-infection.

30

Using infectious material derived from tissue samples collected during disease

investigation in the field, and subsequent passage in vitro, we were able to replicate the clinical symptoms observed during the outbreak through both IP and cohabitation infection routes. Transmission of the disease from IP infected to cohabitant naïve fish in the same water space was also demonstrated, confirming the infectious nature of this pathogenic agent.

Example 4: Sample preparation, tissue homogenization, DNA isolation of tissue samples collected during the infection experiment (Example 3)

Homogenization of Organ Samples

Fish organ samples collected in the experiment described above (Table 4, 5) were homogenized using a Precellys 24 Homogenizer instrument. A 10% organ homogenate was made in Phosphate Buffered Saline (PBS) using a program existing of two cycles of 20 seconds at 6,500 rpm with a 10 seconds interval. Homogenization of heart, spleen, kidney, brain, intestine and liver samples was done in one cycle, skin and gill samples were homogenized twice. All samples were kept on ice during homogenization and were stored at -80°C.

DNA Extraction

DNA extraction was performed using a MagNA Pure 96 System and a MagNA Pure 96 DNA and Viral NA Kit. For extraction, 250 µl MagNA Pure 96 External Lysis Buffer was added to 200 µl sample. DNA was isolated with a pre-installed external lysis protocol and eluted in 50 µl Milli-Q water. DNA was stored at -20°C until further use.

Example 5: Culture of the virus and titration of the virus

Establishment and culture of Seabass Brain (SBB) cell line:

The cell line SBB was originally derived at Intervet Norbio Singapore Pte Ltd (part of MSD AH) from a trypsinized suspension of Asian Seabass brain cells. Procedures for derivation of Seabass Brains cells have been described by Hasoon et al., in In Vitro Cell. Dev. Biol. – Animal 47: 16-25 (2011) and by Chi et al. Dis Aquat Organ. 2005 Jun;65(2):91-8.

The SBB culture medium consists of 899 ml E-MEM supplemented with 2 mM L-glutamine and 110 mg/L sodium pyruvate, 100 ml FCS (10%) and (optional) 1 mL of a Neomycin Polymyxin antibiotics solution 1000x stock. Cells were routinely grown at
5 28°C and 5% CO₂.

The culture medium was kept at 4°C prior to startup of a culture. One ampoule of frozen stock SBB was used to start a culture. Cells from liquid nitrogen were thawed fast by warming ampoule in 28°C water. The cell suspension was added to a tube and diluted
10 slowly with 9 volumes of culture medium. Subsequently, cells were counted. The suspension was dispensed to the appropriate culture flask or roller bottle and incubated at 28°C and 5% CO₂. Seeding density in the flask or roller bottle was approximately 3×10^4 cells / cm². After 6-24 hours or complete attachment of the cells, the culture medium was refreshed to remove the remaining DMSO (freeze medium consists of 90% culture
15 medium and 10% DMSO). The cells were further incubated for 3-7 days or until confluence was reached. For roller bottles, a roller speed of 0.2-0.5 rpm was required. Roller bottles can be of different surface areas of 480, 960 and 1750 cm².

Once confluence was reached, cells were passaged. The passage can be performed
20 every 3-4 days with an initial seeding density of 3.0×10^4 cells / cm². Alternatively the passage can happen every 7 days when plated at a density of 1.0×10^4 cells/cm². The reagents for cell passage (medium, PBS, Trypsin/EDTA) were pre-warmed to 28°C. The culture medium was discarded and the confluent monolayer was washed once with an appropriate volume of PBS (3 mL for a T25 flask). The PBS was subsequently
25 discarded and the cells were incubated in an identical volume of PBS supplemented with 1% (vol/vol) of a 2.5% trypsin solution and 1% (vol/vol) of a 2% EDTA solution for 15 minutes at 28°C. After detachment, an identical volume of fresh culture medium was added and the cells were resuspended and counted. A new flask was set up at the desired cell density in a culture volume appropriate for the culture flask or roller bottle.

30 For freezing cells, culture medium and 2x concentrated freeze medium (80% (vol/vol) culture medium plus 20% (vol/vol) DMSO) were kept at 4°C prior to the procedures. The confluent cell culture was treated as described above up to and including trypsinization. Cells were resuspended, counted, further resuspended in a suitable amount of culture
35 medium, and an equal volume of 2 x freeze medium was added drop by drop while swirling the suspension. Ampoules for liquid nitrogen storage were filled with 5.25×10^6

cells per ampoule to start a T175 or with 2.25×10^6 cells to start a T75.

Inoculation of SBB cells with Lates Calcarifer Herpes Virus

The cells cultured from liquid nitrogen storage were passaged at least once before
5 inoculation experiments were set up. The cells were passaged and cultured 24 hours
before inoculation at
 3.0×10^4 cells/cm² in tissue culture flasks. The inoculum consisted a fresh or freeze-
thawed culture undiluted supernatant from the previous passage of the virus. The
culture medium was removed from the flask. The flask was subsequently inoculated at
10 28°C for at least 60 minutes.

When inoculating cells with a harvest of a previous passage of LCHV virus in culture
medium, preferably a MOI of 0.001-0.01 TCID₅₀ per cell is used.

After the inoculum was removed (after 60 minutes, although this is not an absolute
15 requirement), fresh culture medium was added and cells were cultured until full CPE
was observed using an inverted light microscope (usually after 2-4 days). Virus was
harvested by collection of the culture supernatant which was spun 5 min at 800 x g to
remove debris. Alternatively, the supernatant can be cleared by filtration. Cleared
supernatant was used for a subsequent passage or PCR/DNA/EM analysis or frozen at
20 -70°C. Replication of the virus could be confirmed with (quantitative) PCR analysis
and/or titration of the harvest. DNA sequencing techniques were used to confirm identity
of the virus, as well as EM.

DNA for (quantitative) PCR was isolated from tissue culture medium using the MagNA
25 Pure 96 System and a MagNA Pure 96 DNA and Viral NA Kit (Example 4).

Titration of the virus on SBB cells

SBB cells were cultured as described above. On the day prior to the test, a SBB cell
suspension containing 6.0×10^4 cells/mL in culture medium (EMEM + 10% FCS + L-Glu
30 + NaPyr) was prepared. The 96 wells of a microtiter plate were seeded with 100 µL of
this cell suspension. The plates were incubated for 24 hours at 28°C and 5% CO₂. The
monolayer was about 50% confluent after this incubation period.

At the day of the test, 10-fold serial dilutions of each virus sample were prepared up to
10⁻⁷ by transferring 0.5 mL sample to a tube containing 4.5 mL of cold (0-20°C) titration
35 medium (culture medium with reduced FCS; 50 % EMEM + L-Glu + NaPyr + 50%
Culture medium), mixing and transferring 0.5 mL in a next tube containing 4.5 mL

titration medium, followed by careful mixing, transferring etc. Columns 1 and 12 and rows A and H served as negative control and were inoculated with 100 µL/well fresh titration medium. Microtiter plates were inoculated with 100 µL/well of the virus dilutions 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} in rows B to G (10 wells/dilution). During handling, the temperature of the virus dilutions was kept between 0°C and 20°C. The plates were incubated at 28°C and 5% CO₂ for 6 days. After the 6 day virus incubation period the plates were screened for LCHV specific CPE with an inverted light microscope. The CPE was characterized by rounding up of cells, followed by cell detachment/lysis (Figure 2). Each well that shows LCHV specific CPE was scored as positive. TCID₅₀ was determined according to the method and calculations described by Reed and Muench, *am. J. Epidemiol.* (1938) 27(3): 493-497. qPCR analysis of DNA samples isolated from positive wells in the titration assay confirmed presence and replication of the virus.

15

Results

Tissue culture flasks were seeded at 3.0×10^4 cells/cm² and cultured 24 hours. After 24 hours, cells in one flask were counted after trypsinization to determine the actual number of cells present in the flask. The culture medium was removed from the other flasks that were subsequently inoculated. Inoculum at 0.001 TCID₅₀ per cell consisting of an undiluted culture supernatant from a previous passage of LCHV virus in culture medium (passage number of the virus between 4-8) was applied to the monolayers and incubated for 60 minutes. The inoculum was removed and fresh culture medium was added to the cell culture flask. Cells were cultured until full CPE was observed using an inverted light microscope. Virus was harvested by collection of the culture supernatant which was spun 5 min at 800 x g to remove debris. Samples were taken from (1) the undiluted inoculum used for infection of the monolayer, (2) the culture supernatant of a flask harvested 1 hour after replacing the inoculum with fresh medium, (3) a monolayer at 50% CPE and (4) a monolayer at 100% CPE. Titrations were performed on samples (1), (3) and (4), and qPCR analyses were performed on samples (1), (2), (3) and (4). The results are presented in Table 8. Pictures were captured at a 40 x magnification using an Olympus CKX41 inverted light microscope. These are shown in figure 2. Figure 2A shows morphology of SBB cells (p18) at 90% confluence and figure 2B at 50% CPE in a culturing flask.

35

Table 8: Detailed results LCHV growth on SBB cells.

Sample	LCHV TCID ₅₀ /mL			LCHV copies/mL
	Tit. 1	Tit. 2	Average	
(1) Inoculum	3.5	3.4	3.4	2.5×10^7
(2) 1h after medium replacement	NA*	NA*	NA*	3.5×10^5
(3) 50% CPE	6.7	6.7	6.7	2.8×10^9
(4) 100% CPE	6.6	7.2	6.9	5.4×10^9

*NA: not analyzed

5 **Example 6: Electron microscopy**

Electron Microscopy

Copper grids of 400 mesh with a pure carbon film were exposed for 20 sec to a glow discharge in air to make the film surface hydrophilic. Virus samples was placed on the carbon-coated grid with a volume of 10 µl and left to incubate for approximately 2 min. Excess sample was blotted with a filter paper and 10 µl of water was placed on the grid and immediately removed again by blotting. Then 10 µl of 1% uranyl acetate was placed on the grid for staining. After 30 seconds, excess stain was removed by blotting and the specimen was left to dry for a few minutes before viewing in the electron microscope. Specimens were observed in a JEOL 1011 transmission electron microscope operating at 80 kV. Images were recorded using a SIS Veleta 2kx2k camera.

Pictures of LCHV culture samples were captured using a JEOL 1011 transmission electron microscope to confirm that the identified virus is a Herpes virus and to exclude the presence of any other viruses. SBB (p9) cells were inoculated with LCHV passage 5 at an MOI of 0.01. After harvesting virus was stored in culture medium at -80°C and a titer of $10^{4.43}$ TCID₅₀/mL was obtained. A 1 µl sample was prepared for electron microscopy of which two pictures are shown in Figure 3. In Panel A, two dark spots can be observed with an approximate diameter of 100 nm, matching the average diameter of the capsid of common Herpes viruses (115-130 nm). Panel B shows a magnification in which the icosahedral contour of this particle can clearly be observed. No enveloped viruses were found in the sample.

Figure 3 shows the LCHV virus captured with an Electron Microscope. In figure 3A two Herpes viruses (dark spots) are recognizable. The scale bar is 500 nm. Figure 3B shows a magnification of one of the spots, clearly showing an icosahedral contour.

- 5 The examples presented above describe detection and isolation of a novel pathogen from diseased fish. The identical disease symptoms could be reproduced after experimental infection of healthy fish with the isolated pathogen. The infectious agent isolated from the experimentally infected fish was identical to the pathogen that was initially isolated. This is proof that the disease symptoms described above are solely
10 attributable to the pathogen that was discovered, Lates Calcarifer Herpes Virus.

Example 7: primary cell culture Seabass fin cells

- 15 A primary cell culture from seabass fin cells (SBF) was established. Cells were cultured for at least 5 passages in culture.
- The culture was established as follows. Fish were anesthetized. The caudal fin (tail) was trimmed and washed three times in PBS + gentamicin 0.3% + enrofloxacin 0.002% +
20 amphotericin 0.5%. The fins were cut into tissue fragments using a scalpel blade. The fragments were transferred into 25 cm² tissue culture flasks containing L15 culture medium supplemented with 20% FCS and gentamicin 0.3% + enrofloxacin 0.002% + amphotericin 0.5%. The flask was incubated at 28°C in a humidified incubator without CO₂. The medium was changed (L15) as required based on presence of debris and pH.
- 25 Cells were passaged by trypsinisation with 0.125% trypsin in PBS till cell detachment and split at low ratio between 1: 1-3, depending on cell density. During the initial passages cells were cultured in L15 medium supplemented with 20%FCS. The percentage FCS was reduced to 10% in later passages.
- 30 Inoculation with Lates Calcarifer Herpes virus was carried out using procedures as described in Example 5. Day 4 post infection 100% CPE was observed.

Example 8: Additional strain of the novel virus

- 35 Samples of sick fish were obtained from a farm, different from the index farm, where clinical symptoms of Lates Calcarifer Herpes Virus infection were observed. However,

PCR analysis using the primer set 2 (SEQ ID NO:17 and SEQ ID NO:18) described in Example 2 gave a negative PCR result. As an alternative, primer sets designed based on the Polymerase (ORF21) and Terminase (ORF 37) sequence, ORFs with relatively high levels of amino acid conservation compared to other *Alloherpesviridae*, were used
5 for PCR. The primers used for PCR are presented in Table 2d and 2e.

These PCRs gave positive results. The Terminase PCR fragment was sequenced and showed 97% identity with the sequence of Lates Calcarifer Herpes Virus as presented in SEQ ID NO:1. SEQ ID NO:29 presents the PCR product of the Terminase PCR of
10 LCHV as presented in SEQ ID NO:1. SEQ ID NO:30 presents the PCR product of the Terminase PCR of LCHV obtained from the farm different from the index farm. It was concluded that the outbreak of disease was caused by another strain of LCHV.

PCT

0-1	Form PCT/RO/134 Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)	
0-1-1	Prepared Using	CMS Online Filing Version CMS 1.14 MT/FOP 20020701/0.20.5.20
0-2	International Application No.	
0-3	Applicant's or agent's file reference	24348 WO
1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
1-1	page	page 3 and 7 and 34
1-2	line	29 and 16 and 21
1-3	Identification of deposit	
1-3-1	Name of depositary institution	CNCM Collection nationale de cultures de micro-organismes (CNCM)
1-3-2	Address of depositary institution	Institut Pasteur 25-28, rue du Dr. Roux 75724 Paris Cédex 15, France
1-3-3	Date of deposit	28 July 2016 (28.07.2016)
1-3-4	Accession Number	CNCM I-5118
1-4	Additional Indications	
1-5	Designated States for Which Indications are Made	All designations
1-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	

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0-4	This form was received with the international application: (yes or no)	yes
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CLAIMS

1. An isolated herpesvirus which is a member of the family of Alloherpesviridae, and which in its wild-type form causes a disease in Asian sea bass which is characterised by the following signs: onset of clinical signs around 3 days after challenge, generalized skin lesions leading to darkened skin with pale patches and fin erosions, lethargy with observed loss of swimming equilibrium, inappetence, increase in opercular respiration rate, and occurrence of mortalities about 2 weeks after challenge.
2. An isolated herpes virus according to claim 1, characterised in that the virus has DNA that over its full length is at least 70% identical to the DNA having a sequence according to SEQ ID NO:1.
3. A isolated herpes virus according to claim 2, characterised in that in the DNA of the virus, at least 95% of the open reading frames have at least 80% sequence identity with the corresponding open reading frames of the DNA having a sequence according to SEQ ID NO:1.
4. An isolated herpesvirus which is a member of the family of Alloherpesviridae, which virus has at least one of the identifying characteristics of the virus deposited under Accession Number **CNCM I-5118** at the Collection Nationale de Cultures de Microorganismes (CNCM), Institut Pasteur, Paris , France.
5. An isolated herpesvirus according to claim 4, characterised in that the identifying characteristics are chosen from the group consisting of:
 - the virus having a DNA sequence that over its full length is at least 70% identical to the sequence according to SEQ ID NO:1;
 - the virus in its wild type form causes disease in Asian sea bass which is characterised by the following signs: onset of clinical signs around 3 days after challenge, generalized skin lesions leading to darkened skin with pale patches and fin erosions, lethargy with observed loss of swimming equilibrium, inappetence, increase in opercular respiration rate, and occurrence of mortalities about 2 weeks after challenge;
 - the virus comprising a Major Envelope Protein (MEP) gene having a level of identity of at least 80% to the nucleotide sequence as depicted in SEQ ID NO: 2;

- the virus comprising a dUTPase gene having a level of identity of at least 80% to the nucleotide sequence as depicted in SEQ ID NO: 4;
- the virus comprising a Terminase gene having a level of identity of at least 80% to the nucleotide sequence as depicted in SEQ ID NO: 6;
- 5 - the virus comprising a Polymerase gene having a level of identity of at least 80% to the nucleotide sequence as depicted in SEQ ID NO: 8.

6. An isolated herpes virus which is a member of the family of Alloherpesviridae, and which in its wild-type form causes a disease in Asian sea bass, characterised in that
10 virus has DNA that over its full length is at least 70% identical to the DNA having a sequence according to SEQ ID NO:1.

7. An isolated herpesvirus which is a member of the family of Alloherpesviridae, and which in its wild-type form causes a disease in Asian sea bass, characterised in that the
15 virus has DNA wherein at least 95% of the open reading frames have at least 80% sequence identity with the corresponding open reading frames of the DNA having a sequence according to SEQ ID NO:1.

8. An isolated herpesvirus comprising a Major Envelope Protein (MEP) gene,
20 characterized in that:

- a) the virus is a member of the family Alloherpesviridae
- b) the virus causes disease in Asian sea bass
- c) the nucleotide sequence of the MEP gene has a level of identity of at least 80% to the
25 nucleotide sequence as depicted in SEQ ID NO: 2.

9. An isolated herpesvirus comprising a dUTPase gene, characterized in that:

- a) the virus is a member of the family Alloherpesviridae
- 30 b) the virus causes disease in Asian sea bass
- c) the nucleotide sequence of the dUTPase gene has a level of identity of at least 80% to the nucleotide sequence as depicted in SEQ ID NO: 4.

10. An isolated herpesvirus according to claim 8 or 9, wherein the virus comprises the
35 Major Envelope Protein gene and the dUTPase gene characterized in that the nucleotide sequence of the MEP gene has a level of identity of at least 80% to the

nucleotide sequence as depicted in SEQ ID NO: 2 and the nucleotide sequence of the dUTPase gene has a level of identity of at least 80% to the nucleotide sequence as depicted in SEQ ID NO: 4.

- 5 11. An isolated herpesvirus comprising a Major Envelope Protein (MEP) gene, characterized in that:
- a) the virus is a member of the family Alloherpesviridae
 - b) the virus causes disease in Asian sea bass
 - 10 c) the MEP gene reacts in a PCR reaction with a primer set as depicted in SEQ ID NO: 21 and 22 to give a PCR product of 277 +/- 10 base pairs.
12. An isolated herpesvirus comprising a dUTPase gene, characterized in that:
- 15 a) the virus is a member of the family Alloherpesviridae
 - b) the virus causes disease in Asian sea bass
 - c) the dUTPase gene reacts in a PCR reaction with a primer set as depicted in SEQ ID NO: 23 and 24 to give a PCR product of 346 +/- 10 base pairs.
- 20 13. An isolated herpesvirus comprising a Terminase gene, characterized in that:
- a) the virus is a member of the family Alloherpesviridae
 - b) the virus causes disease in Asian sea bass
 - c) the Terminase gene reacts in a PCR reaction with a primer set as depicted in SEQ ID
 - 25 NO: 25 and 26 to give a PCR product of 585 +/- 10 base pairs.
14. An isolated herpesvirus comprising a Polymerase gene, characterized in that:
- a) the virus is a member of the family Alloherpesviridae
 - 30 b) the virus causes disease in Asian sea bass
 - c) the Polymerase gene reacts in a PCR reaction with a primer set as depicted in SEQ ID NO: 27 and 28 to give a PCR product of 314 +/- 10 base pairs.
15. A DNA fragment comprising a gene encoding a Major Envelope Protein
- 35 characterized in that said gene has a level of identity of at least 80% to the nucleotide sequence of the MEP gene as depicted in SEQ ID NO: 2.

16. A Major Envelope Protein characterized in that said MEP has an amino acid sequence that is at least 70% identical to the amino acid sequence of SEQ ID NO:3.
- 5 17. A DNA fragment comprising a gene encoding a dUTPase characterized in that said gene has a level of identity of at least 80% to the nucleotide sequence of the dUTPase gene as depicted in SEQ ID NO: 4.
- 10 18. A dUTPase characterized in that said dUTPase has an amino acid sequence that is at least 70% identical to the amino acid sequence of SEQ ID NO:5.
- 15 19. DNA fragment having an open reading frame having a length of at least 100 nucleotides, wherein the DNA has at least 80% sequence identity with an open reading frame of the DNA that has a sequence according to SEQ ID No. 1.
20. A protein encoded by the DNA fragment of claim 19.
21. A cell culture comprising a replication competent virus, characterized in that said culture comprises the herpesvirus according to any of claims 1-14.
- 20 22. A vaccine for combating herpesvirus disease in fish, characterized in that said vaccine comprises a herpesvirus according to any of claims 1-14, or a protein according to any of the claims 16, 18 and 20, and a pharmaceutically acceptable carrier.
- 25 23. A vaccine according to claim 22, wherein the vaccine comprises a herpesvirus according to any of claims 1-14, characterized in that said herpesvirus is in a live attenuated or inactivated form.
- 30 24. A method of prophylactically treating an animal with a vaccine according to claim 22 or 23, comprising systemically administering the vaccine to the animal.
25. An antibody or antiserum reactive with a virus according to any of claims 1-14.
- 35 26. A diagnostic test kit for the detection of antibodies reactive with a virus according to any of claims 1-14 or with antigenic material thereof, characterised in that said test kit comprises a virus according to any of claims 1-14 or antigenic material thereof.

27. A diagnostic test kit for the detection of a herpesvirus according to any of claims 1-14 or antigenic material thereof, characterised in that said test kit comprises antibodies reactive with a virus according to any of claims 1-14 or with antigenic material thereof,
- 5 or a PCR primer set as defined in any of the claims 11-14.