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EMIDA Call Progress Report

(FINAL)

Project Title: Molecular Tracing of viral pathogens in Aquaculture
Acronym: MOLTRAQ
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1. Summary of the project's progress

Consider each of the following items:

- *Main activities and achievements of the consortium*
- *Your opinion on the internal cooperation and added value to the project*

Please state if developments within the projects or outside have caused you to amend any of the project's goals and, if so, in what way

One of the main aims of the project was to assemble molecular and epidemiological information on virus isolates and virus sequences available in the virus collections of the participating partners. Thus, an overview of specimens available within the project group was made and has subsequently been used to select sequences suitable for studies of evolutionary relationships among groups of virus isolates, and for full-genome sequencing (determining the complete DNA or RNA sequence of virus' genome). In addition, detailed information on each specific isolate and/or sequence have been uploaded to the databases in the http://www.fishpathogens.eu website for those virus families where databases were already in place and working (Viral Hemorrhagic septicaemia virus (VHSV) and Infectious haematopoietic necrosis virus (IHNV)). A database on betanodaviruses has also been developed and published during the project period, and work has started on salmonid alphaviruses.

The composition of the project group and the combined skills and expertise of the different partners has allowed for phylogenetic comparisons of differences and similarities between virus isolates over a wide span in time and space:

- Nearly 400 European isolates of VHSV have been partially sequenced (glycoprotein gene) and compared with 200 others found in Genbank.
- About 50 isolates of eel rhabdoviruses (AngRV) from various European countries have been partially sequenced (genes N, P and G)
- Full-length sequencing data have been obtained by deep sequencing 19 samples of Cyprinid herpesvirus 3 (CyHV3) (after successful viral DNA enrichment) and 10 samples of ostreid herpesvirus-1 (OsHV-1).
- Nearly 100 specimens of OsHV-1 collected in different geographical locations (Europe, America, Asia and Oceania) have been partially sequenced (3 differents areas of viral genome) and compared with around 80 others mainly collected in France.
- Sequencing of 63 European IHNV isolates (glycoprotein gene) has been achieved.
- Sequencing of a set of betanodavirus from various outbreaks in the Mediterranean has been completed (partial RNA 1 and RNA2).

The intraspecific phylogeny and evolution of VHS on a European base has been elucidated. Furthermore, the large amount of isolates sequenced has allowed for a much higher resolution of the isolates, making it possible to divide them into further subgroups. These subgroups show that only one VHSV subgroup of closely related isolates survived until VHSV became eradicated in Denmark, providing important information about the eradication process in Denmark.

For CyHV3, phylogenies on full-length genomes revealed a very low diversity (> 99.98% of sequence identity) and confirmed the existence of two main lineages; one Asian and one European. However, some atypical samples exhibited a marked divergence (~3%), which raises the question whether they constitute a new species within the Alloherpesviridae family.

Isolates and specimens have been exchanged between project partners to enable this, and also external partners have provided valuable biologic material and gained access to some of the project data in return.

The genetic diversity of OsHV-1 that has been ravaging the Pacific Oyster industry in later years has also been disentangled through the collaboration with another project (Bivalife).

Molecular tracing of outbreaks of VHS, IHN and Viral Nervous Necrosis (VNN) caused by betanodaviruses has been performed and manuscripts for publication of the findings are currently under preparation.

Further, studies on evolutionary relationships of Eel rhabdoviruses, oyster herpesvirus and betanodaviruses have been performed and published.

Another achievement of the project was towards identification of molecular markers involved in virus spread or virulence. Determining how OsHV-1 and adult oyster interact is a major goal in understanding why mortality events are not reported among adult oysters. Work performed under the project suggests that over-expression of one of the host-genes could be a reaction to OsHV-1 infection, which may induce an apoptotic process. Apoptosis could be a main mechanism involved in disease resistance in adults.

Next-generation sequencing was used to investigate miRNAs (small, non-coding RNA which functions in regulation of gene expression) in CyHV-3. This resulted in the identification of more than 80 potential miRNAs. These predicted miRNAs are spread all along the CyHV-3 genome. All of these putative miRNAs apparently have a unique sequence in CyHV-3 genome, and some of them seem to be conserved in closely-related herpesviruses such as CyHV-1, CyHV-2 or Anguillid HV-1.

These results lay the basis for future investigations aiming at understanding how these potential virulence factors participate in the spread and/or reactivation of the virus in vivo. Further, these miRNAs can possibly be used in designing a diagnostic tool for identifying infection with CyHV-3 in healthy carriers.

In addition to these studies on evolution and virulence, the project aimed at discovering the means of transmission of viruses though the combination of molecular analysis and epidemiological studies.

A model has been developed and published for the spread of salmonid alphaviruses in Norway. The model has subsequently been modified and applied on a corresponding study on transmission of VHSV in freshwater aquaculture, thus demonstrating the genericity of the model.

Finally, the model has been combined with an economic model to explore the economic consequences of different intervention strategies.

Within the project, five physical project meetings have been held: A kick-off meeting, progress meeting 1-3 and a progress meeting specifically for two of the workpackages.

In addition, two workshops have been held: An internal workshop for project partners on spatio-temporal modelling and phylogenetic and evolutionary analysis and an open workshop on molecular tracing of viral diseases in aquaculture, which included also a stakeholder meeting where the results of the project were presented.

A total of 11 manuscripts presenting project results have been published in peer reviewed journals, and 9 more are in the pipeline. Four oral presentations and three posters were presented at the 16th International conference on Diseases in Fish and Shellfish in Tampere, Finland in September 2013, and at the 17th International conference on Diseases in Fish and Shellfish in Las Palmas in September 2015 5 oral presentations will be given, in addition to a dedicated workshop presenting and discussing the main findings of the project. A total of 33 oral presentations and 3 posters were given with reference to MOLTRAQ at international meetings and conferences

At the project homepage: http://moltraq.wordpress.com news on the project and publications are presented.

2. Achievement of planned objectives

Describe the activities that have been performed to meet the objectives set in the proposal.

The overall objectives of the project were:

1. To collect isolates of specific important fish and mollusc viruses and their respective epidemiological data
2. To identify all the isolates by phylogenetic analysis and select isolates of special interest for gene expression studies
3. To identify important factors affecting the spread of virus diseases in aquaculture
4. To construct scenario simulation models to assess effects of different control strategies

(For a complete description of each Milestone as referred to below, please see attached Summary of workpackages and GANTT diagrams)

Towards objective 1, the following activities have been performed:

Milestone M2.1: Sample collections. Community samples collected and ready to use for the group.

143 of 146 VHSV samples were propagated in cell cultures. A specific protocol for KHV sequence capture by hybridization has been designed and tested on a total of 8 KHV specimens (7 from Indonesia and 1 from Poland). OsHV-1 samples from different geographical locations have been included in the collection (from France and other countries including Australia, Brazil, Japan, Korea, Mexico, New Zealand, Spain, the Netherlands, Ireland, Tunisia, UK, USA).

Degree of completion: Completed

Milestone M2.2: Sequences of genomes of our collection

The full length glycoprotein gene of 715 VHSV and 213 IHNV isolates were collected in a database at FLI. The collected sequences originated from published data (150 IHNV G genes and 407 VHSV) as well as from identified sequences at FLI. Approximately 145 Danish, 3 Dutch, 14 Swiss and 50 German VHSV and 12 Dutch, 8 Swiss and 16 German IHNV were propagated on cell culture. 75 Danish VHSV isolates have been partially sequenced (G-gene) at DTU and more than 300 sequences from Germany, Denmark and the rest of Europe have been partially sequenced (G-gene) sequenced at FLI. Sequencing of 63 European IHNV isolates (glycoprotein gene) has been achieved by FLI and IZSVe. All analyzed isolates are genetically related to known European VHSV and IHNV respectively.

About 50 isolates of AngRV have been partially sequenced (genes N, P and G), showing a low diversity at the European scale.

Partial sequencing (partial RNA 1 and RNA2) of a small set of betanodavirus from a recent outbreak in the Mediterranean has been completed.

About 100 OsHV-1 specimens collected in different geographical locations (Europe, America, Asia and Oceania) have been partially sequenced (ORF 4, ORFs 35/36/37/38 and ORFs 42/43) and compared with around 80 others mainly collected in France. DNA (total DNAs) of good quality have been obtained from 12 OsHV-1 samples for full genome sequencing.(Illumina). Full genome sequences have been analysed. Moreover, a genotyping approach was developed targeting 10 microsatellite loci and used to analyze OsHV-1 specimens. The results obtained using genotyping were compared to the results obtained through partial sequencing.

Full-length sequencing data have been obtained by deep sequencing 19 samples of CyHV3 after successful viral DNA enrichment. The strategy for specific enrichment of CyHV-3 genomes was first tested on 8 specimens, among which 7 from Indonesia. Even though the rate of enrichment was directly correlated to the initial viral load, results revealed that full

genomes could be recovered from gill samples containing as little as 5,000 CyHV-3 copies, with a high depth (>100x) almost all along the genome. Subsequently, another 19 CyHV-3 specimens or isolates from all over the world were enriched and sequenced. These latter included specimens that could not be detected by the OIE-recommended primers and/or that did not elicit the clinical signs classically associated with KHVD (provided by FLI and by the Central Veterinary Institute of Wageningen). From these, 10 full sequences and 9 nearly-full or partial genomes (atypical specimens) could be recovered.

Degree of completion: Fully completed

Milestone M2.3: Collating and systematize epidemiological data

All isolate and sequence information on VHSV collated and sequenced during the MOLTRAQ project has been uploaded to www.Fishpathogens.eu. All reports will be publically available after all relevant articles have been published. A new database for nodavirus has been established for Fishpathogens.eu with sequence and isolate information on 59 isolates and a paper has been published on this database. A new database for SAV is under development.

Degree of completion: Completed

Towards objective 2, the following activities have been performed:

Milestone M3.1: Nucleic acid alignments and phylogenetic trees

More than 200 Danish VHSV isolates from 1970-2009 has been aligned and subjected to phylogenetic analysis, including Bayesian and Maximum Likelihood analysis. Results show that most of the Danish isolates belong to the Ia genotype, but a small collection of isolates from farmed rainbow trout is located in the Ic genogroup, a group more closely associated with marine fish. Furthermore, the large amount of isolates sequenced during the MOLTRAQ project allows a much higher resolution of the Ia isolates, making it possible to divide the Ia isolates into further subgroups. These subgroups show that only one VHSV subgroup of closely related isolates survived until VHSV became eradicated in Denmark, providing important information about the eradication process in Denmark.

For KHV, phylogenies on full-length genomes revealed a very low diversity (> 99.98% of sequence identity) and confirmed the existence of two main lineages, i.e. one Asian and one European. However, some atypical samples exhibited a marked divergence (~3%), which raises the question whether they constitute a new species within the Alloherpesviridae family.

In addition, phylogenetic analysis of the following viruses have been performed and published: AngRV (Bellec et al), CyHV3 (Hamoumi et al., submitted), OsHV-1 (Renaut et al.), betanodaviruses (Kara et al; Mikkelsen et al.), VHSV (Cieslak et al.)

Degree of completion: Fully completed

Milestone M3.2: Accurate genetic classification of viral isolates Activities: Accurate species/genogroup classification of all the studied viruses.

Degree of completion: Fully completed

Towards objective 3, the following activities have been performed:

Milestone M4.1: Design of oligonucleotides.

For OsHV-1, a set of 39 primer pairs was designed and validated by real-time RT PCR for virus RNA detection and quantification. OsHV-1 genes were selected based on protein functions or structures of related proteins among the 124 ORFs of OsHV-1 and belong to 5 groups/families:(i) genes encoding enzymes or proteins presenting known viral domains, (ii) inhibitors of apoptosis, (iii) RING-finger genes, (vi) genes predicted to encode membrane proteins and (v) 7 ORFs encode unknown proteins.

For KHV, a search for potential microRNAs (or miRNAs) was carried out on the 3 published genomes. Since nearly 50 sequence motives were identified as potential miRNAs, it was decided to perform a pre-screening by massively sequencing the micro-transcriptome of infected cell cultures.

Degree of completion: Completed

Milestone M4.2: Validation of the oligonucleotide arrays

Since qPCR and NGS approaches were used, this milestone was no longer relevant (see "problems and changes in objectives").

Degree of completion: Not relevant

Milestone M4.3: Identification of molecular markers involved in virus spread or virulence

For OsHV-1, an in vivo transcriptomic study targeting 39 genes was conducted during an experimental infection of the Pacific oyster spat, Crassostrea gigas. The expression kinetics of these genes was monitored at 0h, 2h, 4h, 18h, 26h and 42h post infection (pi). A few transcripts were detected as soon as 2h pi, all others at 18h pi. OsHV-1 DNA was detected earlier than any viral RNA in experimentally infected oysters. The regulation of host genes towards infection is now being studied in two different oyster families, which appears to be differently regulated. Additonally, a study was carried out in adult oysters. Adult oysters do not demonstrate mortality in the field related to OsHV-1 detection and are thus assumed to be more resistant to viral infection. Determining how virus and adult oyster interact is a major goal in understanding why mortality events are not reported among adult oysters. Dual transcriptomics of virus-host interactions were explored by real-time PCR in adult oysters after a virus injection. Thirty-nine viral genes and five host genes including MyD88, IFI44, IkB2, IAP and Gly were measured at 0.5, 10, 26, 72 and 144 hours post infection (hpi). No viral RNA among the 39 genes was detected at 144 hpi suggesting the adult oysters are able to inhibit viral replication. Moreover, the IAP gene (oyster gene) shows significant up-regulation in infected adults compared to control adults. This result suggests that over-expression of IAP could be a reaction to OsHV-1 infection, which may induce the apoptotic process. Apoptosis could be a main mechanism involved in disease resistance in adults. Finally, an in situ hybridization protocol for detecting mRNAs of ostreid herpesvirus type 1 (OsHV-1) which infects Pacific oysters, Crassostrea gigas, was developed. Three RNA probes were synthesized by cloning three partial OsHV-1 genes into plasmids using three specific primer pairs, and performing a transcription in the presence of digoxigenin dUTP. The RNA probes were able to detect the virus mRNAs in paraffin sections of experimentally infected oysters 26 h post-injection. DNA detection by in situ hybridization using a DNA probe and viral DNA quantitation by real-time PCR were also performed and results were compared with those obtained using RNA probes. The in situ hybridization protocol for detecting mRNAs was also used in order to study the development of the viral infection in experimental conditions. For this purpose, a study was carried out in order to compare OsHV-1 DNA and RNA detection at different time points and localization in experimentally infected oysters using two virus doses: a low dose that did not induce any mortality and a high dose inducing high mortality. Real time PCR demonstrated significant differences in terms of viral DNA amounts between the two virus doses. RNA transcripts were detected in oysters receiving the highest dose of virus suspension whereas no transcript was observed in oysters injected with the low dose.

For KHV, miRNAs were investigated by next-generation sequencing. As a first step, a pre-screening of miRNAs was conducted during a lytic cycle. A CCB cell culture maintained at 20°C was inoculated by a CyHV-3 isolate provided by Anna Toffan from IZSVe. Cells were then collected at 0, 1, 2, 3, 6 and 10 days pi (in duplicate), and small RNAs were extracted and sequenced by NGS. This led to the identification of 8 miRNAs, which number of occurrences significantly increased during the lytic cycle. In order to identify more miRNAs

and investigate their possible involvement in the propagation of KHV, new inoculations of CCB cells with the same Italian isolate were realized at 20°C and 30°C (a non-permissive temperature) and cells were collected at 0h, 2h, 4h, 1 day, 2 days, 3 days, 6 days and 10 days pi (in duplicate). In order to increase the sequencing depth, these 36 samples were sequenced on a larger number of sequencing lanes. This resulted in the identification of more than 80 potential miRNAs. These predicted miRNAs are spread all along CyHV-3 genome, and almost all of them are located inside ORFs. The matching ORFs correspond to either immediate early or early genes. All of these putative miRNAs apparently have a unique sequence in CyHV-3 genome, and some of them seem to be conserved in closely-related herpesviruses such as CyHV-1, CyHV-2 or Anguillid HV-1. Some of the predicted miRNAs showed a high level of similarity with carp sequences, and a few of them matched with regulatory proteins. Count of the number of occurrences of these loci along the lytic cycle indicated a significant increase after 6-10 days pi for nearly 19 of them. Among these latter, 5 showed a particularly high expression level. Yet, none of these 19 candidates could be structurally annotated. Finally, none of these potential miRNAs showed an over-expression in the cells infected at 30°C, confirming a potential role in virulence but not in latency. These results lay the basis for future investigations aiming at understanding how these potential virulence factors participate in the spread and/or reactivation of the virus in vivo.

Degree of completion: Completed

Milestone M4.4: Confirmation of expression profiles by real-time PCR

Since we did not use microarrays, this milestone was no longer relevant (see "problems and changes in objectives").

Degree of completion: Not relevant

Towards objective 4, the following activities have been performed:

Milestone M5.1: Completion of spatio-temporal datasets.

The Norwegian Veterinary institute (NVI) has compiled a complete spatio-temporal dataset on VHS-cases along with the total population of farms at risk in Denmark, including genetic sequences of VHS-case virus. Waterway connectivity between all farms has also been compiled. The data has been fitted to a slightly modified dispersal model on Infectious Salmon Anaemia developed by the Norwegian Computing Central (NR). However, there have been some problems with identifying the proper waterway conectivity between farms, as well as the status of some farms with regard to VHS-infection. This work has therefor been somewhat delayed, but is under progress at the NVI.

Degree of completion: Almost completed

Milestone M5.2: Parameterisation of stochastic models

Fitting the data to a slightly modified dispersal model on Infectious Salmon Anaemia developed by NR has been completed.

Degree of completion: Completed

Milestone M5.3: Development of generic simulation modelling tools

NR has now expanded a previous dispersal model on PD, by including a SIR model on internal dynamics of infection on infected farms. In this model all farms and cohorts over many years are connected relative to location and time, and the rate of infection of farms is modeled as the sum of contributions from different infectious sources. The model has now been adapted to scenario simulation. Simulations from the model on the rate of PD outbreaks when no conditions are changed are compared to simulations where cohorts are removed within a month after an outbreak is observed. This work is now completed and has been published in Preventive Veterinary Medicine.

Degree of completion: completed

Milestone M5.4: Analyses of intervention effects for different host-pathogen systems.

The scenario simulation model on PD dispersal has now been used to produce output from various control scenarios. The simulated interventions comprise of mandatory culling, early detection by screening along with culling, varying complience to the intervention policy and "laissez-faire" policy. The output from the scenario-simulations is incorporated into an economic simulation model to evaluate costs and benefits of various control strategies. The PD dispersal simulation under different control regimes has been completed. The economic simulations and analyses are under progress.

Degree of completion: Partly completed and partly under progress.

In addition to the above mentioned objectives, a workpackage dedicated to the purpose has ensured that the results of the project has been disseminated through the following activities:

Milestone M6.1: Website established

An open website www.moltraq.wordpress.com was established and regularly updated with latest news and events. The website provide information on the project, partners involved, publications etc.

Degree of completion: Fully completed

Milestone M6.2: Relevant media for publication of project aims and objectives identified

A comprehensive list of planned publications in collaboration between the partners was made up and discussed at the first progress meeting. First- and co-authors were agreed upon and a time plan for submission made. Approx. half of these are now published whereas most of the other are submitted or in preparation for publication. Workshop/training courses were likewise planned during each of the progress meetings.The isolate database www.fishpathogens.eu was provided at full disposal for use for the MOLTRAQ project. Isolate information on almost 400 VHSV and IHNV isolates from the period 1977 to 2004 has been uploaded to the database for public use, with sequence information on restricted access until publication of articles written within the MOLTRAQ project.

Furthermore, development of a databases on nodavirus (causative agent of Viral nervous necrosis (VNN) has been finalized and published. In corporation with NVI, a database for SAV is under establishement but not finalized yet under the www.fishpathogens.eu platform. The general structure of the databases has been designed, reference sequences for each virus have been identified and new functional part of the databases have been designed. All isolate and sequence data gathered on SAV have been uploaded to the database.

It was initially discussed to design a database for Koi Herpes Virus (KHV), but due to considerable problems concerning annotation of the virus, it has not yet been possible to establish a database for this virus. A completely new and more user-friendly interface has also been designed to further facilitate the use of fishpathogens.eu.

A list of all presentations given at international meetings and conferences has been uploaded on the webpage

Degree of completion: Fully completed

Milestone M6.3: Workshop on molecular tracing of viral pathogens

An internal workshop was held in Berlin 19th-23rd May 2014 with presentations and practical training and with focus on the following topics:

1.Spatio-temporal modelling of the spread of PD between and within marine fish farms in Norway - with focus on comparing slaughtering strategies by scenario simulations

2.Model for VHSV in Denmark

3.Phylogenic and evolutionary analyses of viral genomes: novel approaches for the study of fish diseases epidemics

4.Molecular epidemiology of salmonid rhabdoviruses collected in northern Italy in the past 20 years

5.Genetic diversity of Anguillid rhabdovirus

6.Genome comparisons: from sequencing to assembly and alignment

7. Introduction to phylogenetic analysis

8.Viral disease susceptibility with regard to the genetic signature of the host population
Degree of completion: Fully completed

Milestone M6.4: Training course on design and use of microarrays

During the project it was realized that the use of microarrays would not provide sufficient added value to the project. The reason was that the tools for sequencing has evolved much faster than anticipated and thereby provided the possibility for much more detailed resolution and in depth study of aquatic viruses. Therefore a workshop "Molecular tracing of viral diseases in aquaculture" was organized instead at UM2, Montpellier, January 26-30, 2015. The workshop was opened with a 1 day open scientific meeting presenting and discussing the outcome of the project to stakeholders, students and scientists. Followed by a 2-day practical training course with the theme "Molecular biology meets epidemiology" and focus on the following topics:

1.Phylogeny of viruses – practical applications in the context of the epidemiology.

2.Practicals in BEAST

3.How may next-generation sequencing help identifying potential virulence factors?

4.How to collate good epidemiological data for molecular tracing.

5.Use of sequence data in epidemiological analysis.

6.Phylogeography.

7.Practicals in epidemiological modelling

The practical training was combined with excellent presentations on "The biography of viral emergence: Rice yellow mottle virus as a case study" by Dr. Denis Fargette, IRD. "Clinical applications of pathogen phylogenies" by Dr. Samuel Alizon, CNRS. "Searching for virus phylotypes" by Dr. Olivier Gascuel, CNRS and "On the origin and tracing of genetic polymorphisms for Picorna-like viruses" by Dr. Gaël Thébaud, INRA. The last progress meeting of the project was held back to back to this training course.

Degree of completion: Fully completed

3. International collaboration added value

Describe the activities that have been accomplished in collaboration within the consortium. Refer explicitly to joint milestones and deliverables produced.
Describe any sharing of facilities, databases within the consortium.

The sharing of isolates led to a valuable enrichment of the phylogenetic data. For example, it has been shown that only rhabdoviruses from the EVEX type are present in Europe (no EVA strain found). This will facilitate the improvement of diagnostic tests. Another example is the impressive genetic data obtained with VHSV isolates which are the most complete ever collated and analysed. This data exclude the presence of the genotype IV in Europe to date and shed light on the dynamic spread of genotype I in the European continent in the last decades.

M2.2/D2b: FLI has sent IRD rare variants of CyHV-3 for whole genome sequencing; they are now analyzing the resulting sequences jointly. Likewise, in the frame of a new collaboration, the laboratory for Fish and Shellfish Diseases of the Central Veterinary Institute of Wageningen has sent IRD several atypical KHV variants that do not elicit any clinical signs in their hosts. These samples have been fully sequenced and sequences are being jointly analyzed. A new collaboration has also been established with the genomics platform of Toulouse (Genotoul), which built a dedicated web portal containing all the KHV sequence data and analyses obtained in the frame of MOLTRAQ. This data is accessible to all MOLTRAQ partners, as well as to other partners upon request.

DTU has sent FLI 145 Danish VHSV isolates for G-gene sequencing. IZSVe has sent to FLI 20 complete G-gene sequences related to Italian VHSV isolates. This sequence information has been included into a large analysis of VHSV in Europe by viral phylodynamics. FLI has sent isolate and sequence information on more than 300 European VHSV isolates to DTU where it has been uploaded to Fishpathogens.eu and which will be available freely for public dissemination.

M4.3/D4b: FLI and IRD have built a common protocol for running cell cultures in parallel in order to get duplicate experiments with different strains; IZSVe has sent a 'living' isolate of CyHV3 to IRD for gene expression studies, which revealed very useful considering the difficulties to propagate this virus in vitro.

M5.1. NVI and NR applies their dispersal modelling tools on DTU-Vet epidemiological and genetic data on VHSV.

4. Problems and changes in objectives

Describe any difficulties and problems that have hindered the achievement of the planned objectives and any alternative plans or changes with respect to the original proposal.

WP4: As was decided during the 1st progress meeting, investigation of molecular markers involved in virus spread was conducted on DNA viruses only (KHV and OsHV), as a different strategy would be needed for RNA viruses.

WP4: Considering the recent publications of Ilouze et al. (2012a, b)* describing the expression kinetics of the 156 predicted KHV ORFs, it was decided to increase our focus on the potential role of microRNAs in the virulence, as part of what was originally proposed. This entailed to abandon microarrays and switch to high-throughput sequencing, which seemed the best suited method for studying the expression of microRNAs.

WP5: There have been some problems with identifying the proper waterway conectivity between Danish fish farms, as well as the status of some of the farms with regard to VHS-infection, under milestone M5.1. This work has therefor been somewhat delayed.

*Ilouze M, Dishon A, Kotler M (2012a). Coordinated and sequential transcription of the cyprinid herpesvirus-3 annotated genes. Virus Research 169, 98-106. Ilouze M, Dishon A, Kotler M (2012b). Down-regulation of the cyprinid herpesvirus-3 annotated genes in cultured cells maintained at restrictive high temperature. Virus Research 169, 289-295.

5. Project-derived publications and patents

*Publications **with** the involvement of other partners of the consortium*	See attached list of dissemination
*Publications **without** the involvement of other partners of the consortium*	See attached list of dissemination
*Patents **with** the involvement of other partners of the consortium*	Not relevant
*Patents **without** the involvement of other partners of the consortium*	Not relevant

6. Brief financial report

	1st year	2nd year	3rd year	Total
Personnel	261214	486494	565106	1312814
Equipment	3195	8652	27080	38928
Other costs	104984	171293	247000	523277
Total	369393	666440	839186	1875019

7. Executive summary

The executive summary must not exceed 2 sides in total of A4 and should be understandable to non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

The focus of the project has been to collect virus isolates from several different host-pathogen systems from all over Europe, and utilise these to increase knowledge on transmission, prevention and control of viral diseases in aquaculture and develop a generic approach to viral disease control.
The uniqueness of the MOLTRAQ project was that the focus was on identifying generic properties of viral aquatic pathogens. This could be achieved because the joined project group had a large collection of different virus isolates sampled from many different species of fish and molluscs in many different countries over a considerable timespan.

The background for the project was the compromised efficiency of preventive interventions, that are a result of control strategies for diseases in aquaculture that have been designed based on general knowledge on biosecurity, and only to a very limited degree take into account disease-specific transmission patterns, since the understanding of these are poor.

An overview of achievements
Essential for the project has been the access to high quality data on samples of representative isolates belonging to various fish and shellfish viruses including part- and whole genome sequences together with epidemiological data. This has allowed for phylogenetic comparisons of differences and similarities between virus isolates over a wide span in time and space. Specifically, the intraspecific phylogeny and evolution of one of the most serious aquatic viral diseases, viral haemorrhagic septicaemia virus (VHS), on a European base has been elucidated.
The genetic diversity of the ostreid herpesvirus 1 that has been ravaging the Pacific oyster industry in later years has also been disentangled.
The webbased platform www.fishpathogens.eu has been extended with a database on Betanodavirus, and work on one on Salmonid Alphaviruses has been initiated. The platform ensures worldwide access for professionals and researchers within the field of aquatic viruses, to quality-controlled collections of molecular- and epidemiological data on virus specimens.

Molecular tracing of outbreaks of VHS, Infectious Heamatopoietic Necrosis virus (IHNV) and betanodaviruses has been performed and manuscripts for publication of the findings are currently under preparation.
A model that has been successfully used to trace the outbreaks of Infectious Salmon Aneamia virus in Norway to infections with the low-pathogenic variant HPR0, has been adapted to trace the sources of outbreak of VHS in Denmark, thus demonstrating that the model can be used on several different host-pathogen systems.

In order to gain knowledge into the factors that control viral spread at the molecular level, the project has been investigating the effect of temperature on viral expression for the Carp virus,

Cyprinid herpesvirus-3. For this, high-throughput next-generation sequencing of viral genomes from virus isolates collected at different temperatures has been performed, thus using this method for identifying potential virulence factors.

Finally, a stochastic model using spatio-temporal data on infected and non-infected populations of Atlantic Salmon in Norway, simulating the spread of Pancreas Disease (PD) has been developed. This model is now being used to investigate the economic consequences of using screening for virus to forecast disease-outbreaks and perform coordinated early harvest to avoid outbreaks in defined zones.

MOLTRAQ and the future

The extensive sequencing of many isolates of a given viral species has opened a path to improve existing or propose new diagnostic methods for some viral species. The data provides a large vision of the sequences that are very conserved between strains (best target for detection) and also the sequences that are variable (best targets for tracing the origins of an outbreak). For instance, the picture of the variabilities of two very different models, AngRV (less variable than expected) and CyHV3 (more variable than expected), will allow the design of new diagnostic tests that are more specific and hopefully more sensitive.
In addition, molecular specificities (‘markers’) have been found in some isolates within both species (for instance, P gene for AngRV). It will be of high interest to observe the evolution of these markers in the coming years, either to visualize their geographic spread or to explain the potential evolution of their virulence.
As microRNAs that can detect healthy carps that are carrying CyHV-3 has been discovered during the project, this could be explored as a tool used for screening in order to avoid spread of this virus.
The number of identifications of nodavirus, the causative agent of VNN (Viral nervous necrosis) from wild fish is rapidly growing and the disease is apparently spreading in the Mediterranean area. Further molecular analysis will reveal farm-wild life interactions and spreading mechanisms. The Nodavirus database established within the project will facilitate this study.

During MOLTRAQ, we have explored the possibility to adapt scenario simulation models and models for molecular tracing for spread of viral diseases in aquaculture to different viral-pathogen systems. It has been proven feasible to use the same models to different settings, and also for testing different intervention strategies. This will be explored further in the future, and we expect this can be an important tool for advising how to control viral diseases in aquaculture.

Thanks to MOLTRAQ the repository of sequences fromViral haemorrhagic septicaemia virus (VHSV) and infectious hematopoietic necrosis (IHNV) is very significant and will be used in future tracing of new outbreaks in Europe. The increasing number of full genome sequences will as well be a tool for assessing virulence markers, and designing vaccine components.

All project results have been presented at a stakeholder meeting in Montpellier, France at the end of January 2015, where project partners was also hosting and teaching a workshop on how to use molecular tracing for diseases in aquaculture.

Finally, a workshop discussing the implications and perspectives of the project findings will take place at the 17th International conference on Diseases in Fish and Shellfish in September 2015.

MOLTRAQ was a research-project funded under the EMIDA-ERA Net under the EU 7th Framework program. The project began on April 1st, 2012, and ended on July 31st, 2015. The total budget was 1.9€, of which 1.4€ is funded via the EMIDA-ERA Net.
Project partners were: Norwegian Veterinary Institute (NO), National Veterinary Institute – Tecnical University of Denmark (DK), Agence Nationale de Sécurité Sanitaire (FR), Institut Francais de Recherche pour Léxploitation de la Mer (FR), Institut de Recherche pour le Développement (FR), Friedrich-Loeffler Institut (DE) and Norwegian Computing Centre NO).

You can read more and follow the MOLTRAQ-project at: www.moltraq.wordpress.com

It is the intention of the EMIDA consortium to publish the executive summary of the project.

Please confirm your agreement to do so.

YES ☒

NO ☐

If no, please explain:

I declare that the information I have given is correct to the best of my knowledge and belief.

Britt Bang Jensen & Edgar Brun
Name

11.08.2015
Date

Senior Scientist & Head of section
Position held

MOLTRAQ –final report on disseminations (for part 5 of the final progress report)

Published:

1. **Aldrin, M., R. B. Huseby, and P. A. Jansen**. 2015. Space-time modelling of the spread of pancreas disease (PD) within and between Norwegian marine salmonid farms. Prev. Vet. Med., 121: 132-141
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3. **Bellec, L., J. Cabon, S. Bergmann, B. C. de, M. Engelsma, O. Haenen, T. Morin, N. J. Olesen, H. Schuetze, A. Toffan, K. Way, and L. Bigarre**. 2014. Evolutionary dynamics and genetic diversity from three genes of Anguillid rhabdovirus. J. Gen. Virol. 95:2390-2401.
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Submitted or in preparation:

1. **Avarre, J.C., S. Hammoumi, T. Vallaeys, S. Bergmann, C. Klopp, M. Engelsma.** Comparison of CyHV-3genomes of different origins sheds new light on the evolution and propagation of this virus. Frontiers in Microbiology. 2016. In preparation.
2. **Barbosa-Solomieu V., C. Martenot, N. Faury, A. Segarra, M. Housin, S. Webb, A. Joyce, D. Cheslett, Y. Shimahara, I. Paul-Pont, R. Whittington and T. Renault.** Phylogenetic relationships of ostreid herpesvirus 1 samples from different geographical origins. Applied and Environmental Microbiology. In preparation.
3. **Cieslak, M., S.S. Mikkelsen, H. F.Skall, M. Baud, N. Diserens, M.Y. Engelsma, O. L. M. Haenen, V. Panzarin, S. Mousakhani, T. Wahli, N. J. Olesen, H. Schütze.** Epidemiology of Viral Hemorrhagic Septicemia in Farmed Rainbow Trout by Viral Phylodynamics. PLOS Pathogens 2015. Submitted.
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6. **Mikkelsen, S.S., V.Panzarin, A.Fusaro, H.Schuetze, N.J. Olesen.** Molecular tracing of VHS outbreaks in Denmark between 1982 and 2009. Frontiers in Microbiology, 2016. In preparation.
7. **. Renault, T., L. Quintric, N. Faury, G. Tchaleu, A. Segarra, V. Barbose-Solomieu, S. Webb, Y. Shimahara and B. Morga.** Whole genome comparison of OsHV-1 specimens. Journal of General Virology. In preparation
8. **Segarra A., L. Baillon, N. Faury, D. Tourbiez, and T. Renault.** Tissue distribution of the virus OsHV1 following an experimental infection in *C. gigas*. Journal of Virological Methods. Submitted.
9. **Segarra A., and T. Renault.** Evidence of OsHV-1 replication in larval Pacific oysters, *Crassostrea gigas*. Virus Research. In preparation

Abstracts:

1. **Sven M. Bergmann and Heike Schütze,** The Koi Herpesvirus (KHV): Identification and characterization of Cyprinid Herpesvirus 3 (CyHV-3), 17th Annual Meeting of the National Reference Laboratories for Fish Diseases. Copenhagen, Denmark, May 29-30, 2013
2. **Heike Schütze,** IHNV in Europe, 17th Annual Meeting of the National Reference Laboratories for Fish Diseases. Copenhagen, Denmark, May 29-30, 2013
3. **Susie Sommer Mikkelsen, Britt B. Jensen, Peter A. Jansen, Niels-Jørgen Olesen, Laurent Bigarré, Heike Schütze, Svenn M. Bergmann, Tristan Renault, Jean-Christophe Avarre, Magne Aldrin and Edgar Brun**, MOLTRAQ - molecular tracing and epidemiology of viral diseases in aquaculture, 17th Annual Meeting of the NRLs for Fish Diseases. Cph, Dk, May 29-30, 2013
4. **B. Bang Jensen, A.K. Ersbøll, H. Korsholm, H.F. Skall and N.J. Olesen**, Spatio-temporal risk factors for viral haemorrhagic septicaemia in danish aquaculture, 17th Annual Meeting of the NRLs for Fish Diseases. Cph, Dk, May 29-30, 2013

5. **Michael Cieslak & Heike Schütze**, Phylogenetic analyses in fish diseases, 17th Annual Meeting of the NRLs for Fish Diseases. Cph, Dk, May 29-30, 2013
6. **Barbosa-Solomieu, V.**, Faury, N., Joyce, A., Cheslett, D., Webb, S., Renault, T., Phylogenetic relationships of ostreid herpesvirus 1 samples from different geopgraphical origins, 16th International Conference on Diseases of Fish and Shellfish, September 2 - 6, 2013, Tampere, Finland.
7. **L. Bellec, J. Cabon, M. Engelsma,T. Morin, N.J. Olesen, H. Schutze, K. Way, L. Bigarré,** Genetic diversity of anguillid rhabdovirus using n, p and g genes, 16th International Conference on Diseases of Fish and Shellfish, September 2 - 6, 2013,Tampere, Finland
8. **B. Bang Jensen, A.K. Ersbøll, H. Korsholm, H.F. Skall and N.J. Olesen,** Spatio-temporal risk factors for viral haemorrhagic septicaemia in danish aquaculture, 16th International Conference on Diseases of Fish and Shellfish, September 2 - 6, 2013,Tampere, Finland
9. **N.J. Olesen, H.F. Skall, B. Bang Jensen, N. H. Henriksen, S. Mellergård and H. Korsholm**, Eradication of viral haemorrhagic septicaemia in Danish aquaculture, 16th International Conference on Diseases of Fish and Shellfish, September 2 - 6, 2013, Tampere, Finland.
10. **Mikkelsen S. S., Schuetze H, Korsholm H, Bruun M. S, Olesen N.J.** Molecular Tracing of aquatic viruses Moltraq, 18th Annual Meeting of the NRLs for Fish Diseases. Cph, Dk, June 3-4, 2014
11. **Hammoumi S, Santika A, Zainun Z, Vallaeys T, Klopp C, Avarre JC,** Comparative genomics of Cyprinid herpesvirus-3 reveals low genetic diversity, 9th Symposium on Diseases in Asian Aquaculture, Ho Chi Minh, Vietnam, November 24-28, 2014.
12. **N.J. Olesen,** Virus evolution and differentiation in fish farming, 9th Symposium on Viruses of Lower Vertebrates, October 2014, Malaga, Spain
13. **S. S. Mikkelsen, H. Schuetze, H. Korsholm, B. B. Jensen, M. S. Bruun, N.J. Olesen,** Molecular tracing of aquatic viruses – MOLTRAQ, 9th Symposium on Viruses of Lower Vertebrates, October 2014, Malaga, Spain
14. **T. Ito, J. Kurita, K. Mori and N. J. Olesen,** Virulence determinant of viral hemorrhagic septicemia virus genotype III isolate for rainbow trout Oncorhynchus mykiss, 9th Symposium on Viruses of Lower Vertebrates, October 2014, Malaga, Spain
15. **Niels .J. Olesen, Helle F. Skall, Britt B. Jensen, Niels H. Henriksen, Stig Mellergård and Henrik Korsholm,** Control and eradication of viral hemorrhagic septicemia in Danish aquaculture, International Symposium of the American Aquatic Animal Health Society, Portland, US, 31.08-04.09.2015
16. **Bigarré, L., Baud M., Chaoui L., Derbal F., Zaidi R., Kara H,** Spread of viral nervous necrosis on the algerian wild fish in 2012. 9th International symposium on viruses of lower vertebrates, 1-4 october 2014, Malaga.
17. **Cieslak, M**, Bergman, S. M., Schütze, H., A new approach to trace phylogenetically usable neutral sites within a Novirhabdovirus genome to create a time-scaled tree. 9th International symposium on viruses of lower vertebrates, 1-4 october 2014, Malaga
18. **Britt Bang Jensen,** MOLTRAQ - Molecular tracing of viral pathogens in aquaculture, an EMIDA-ERA net project: Background, outline and aims. Workshop "Molecular tracing of viral diseases in aquaculture" UM2, Montpellier, January 26-30, 2015.
19. **Heike Schuetze,** Viral phylogeny and molecular tracing – Infectious hematopoietic necrosis virus as a model. Workshop "Molecular tracing of viral diseases in aquaculture" UM2, Montpellier, January 26-30, 2015

20. **Susie S. Mikkelsen,** Molecular tracing of viral hemorrhagic septicemia outbreaks in Denmark. Workshop "Molecular tracing of viral diseases in aquaculture" UM2, Montpellier, January 26-30, 2015
21. **Sven Bergmann,** *Cyprinid herpesviruses* in European aquaculture. Workshop "Molecular tracing of viral diseases in aquaculture" UM2, Montpellier, January 26-30, 2015.
22. **Jean-Christophe Avarre,** Input of next generation sequencing into viral genome comparisons and analyses: example of *Cyprinid herpesvirus*-3. Workshop "Molecular tracing of viral diseases in aquaculture" UM2, Montpellier, January 26-30, 2015
23. **Tristan Renault,** Describing genetic diversity of *Ostreid herpesvirus* 1 infecting Pacific oysters. Workshop "Molecular tracing of viral diseases in aquaculture" UM2, Montpellier, January 26-30, 2015
24. **Laurent Bigarré.** Tracing fish betanodaviruses. Workshop "Molecular tracing of viral diseases in aquaculture" UM2, Montpellier, January 26-30, 2015
25. **Tatiana Vallaeys,** Development of variable number of tandem repeats (VNTR) based methods to track evolving aquatic herpesviruses. Workshop "Molecular tracing of viral diseases in aquaculture" UM2, Montpellier, January 26-30, 2015
26. **Michael Cieslak,** Intraspecific phylogeny and evolution of the European VHSV lineage Ia. Workshop "Molecular tracing of viral diseases in aquaculture" UM2, Montpellier, January 26-30, 2015.
27. **Britt Bang Jensen,** Scenario simulation models for control options. Workshop "Molecular tracing of viral diseases in aquaculture" UM2, Montpellier, January 26-30, 2015
28. **Anja B. Kristoffersen,** Modelling the spread of pancreas disease (PD) in Norwegian marine salmonid farms. Workshop "Molecular tracing of viral diseases in aquaculture" UM2, Montpellier, January 26-30, 2015.
29. **L. Bigarré, NJ Olesen**, Virus evolution in aquaculture, Workshop "Molecular tracing of viral diseases in aquaculture" UM2, Montpellier, January 26-30, 2015
30. **Susie Sommer Mikkelsen,** MOLTRAQ – Molecular Tracing of VHSV in Denmark. 19th Annual Meeting of the NRLs for Fish Diseases. Cph, Dk, May 27-28, 2015
31. **Sven Bergmann,** Molecular characterization and tracing of KHV. 19th Annual Meeting of the NRLs for Fish Diseases. Cph, Dk, May 27-28, 2015
32. **S. S. Mikkelsen, H. Schuetze, H. Korsholm, B. B. Jensen, M. S. Bruun, N.J. Olesen,** Molecular tracing of VHS in Denmark. 17th International Conference on Diseases of Fish and Shellfish, Canary Islands, Spain September 2015
33. **N. J. Olesen, H.F. Skall, J. Kurita, K. Mori and T. Ito,** Viral haemorrhagic septicaemia virus (VHSV): on the search for determinants important for virulence in rainbow trout *oncorhynchus mykiss.* 17th International Conference on Diseases of Fish and Shellfish, Canary Islands, Spain September 2015
34. **M. Cieslak, M. Baud, N. Diserens, M. Engelsma, O. Haenen, S. Mousakhani, S. S. Mikkelsen, N.J. Olesen, V. Panzarin, H.F. Skall, T. Wahli, H. Schütze,** Genetics of VHSV in Europe. 17th International Conference on Diseases of Fish and Shellfish, Canary Islands, Spain September 2015
35. **L. Bigarré, M. Baud, S. Labrut, M. Jamin and P.-M. Boitard,** VNTR as molecular markers to genotype Cypriniviruses: a review. 17th International Conference on Diseases of Fish and Shellfish, Canary Islands, Spain September 2015.

36. **Hammoumi S, Santika A, Zainun Z, Vallaeys T, Bergmann S, Klopp C, Engelsma M, Avarre JC,** Full-length sequencing and analysis of 25 CyHV-3 specimens reveals atypical genomes with high divergence. 17th International Conference on Diseases of Fish and Shellfish, Canary Islands, Spain September 2015
37. **Hammoumi S, Fourré C, Vallaeys T, Rue O, Gaspin C, Keck N, Avarre JC**, Identification of putative micro-RNAs in CyHV-3 genome and analysis of their expression along a lytic cycle. 17th International Conference on Diseases of Fish and Shellfish, Canary Islands, Spain September 2015

Posters:

1. **B. Bang Jensen, P.A. Jansen, N.J. Olesen, S.S. Mikkelsen L. Bigarré, H. Schuetze, S.M.Bergmann, T. Renault, J.-C. Avarre, M. Aldrin and E. Brun**. Molecular tracing of viral pathogens in aquaculture -a multidisciplinary trans-European research project. 16th International Conference on Diseases of Fish and Shellfish, September 2 - 6, 2013Tampere, Finland
2. **T. Vallaeys, J. Damas, L. Frangeul, N. Berthet, I. Ben Chobba, J.-C. Avarre, T.Renault and A. Gessain**. Comparative genomics of herpes viruses, a paradigm for host parasiteco-evolution. 16th International Conference on Diseases of Fish and Shellfish
September 2 - 6, 2013, Tampere, Finland
3. **V. Panzarin, S.S. Mikkelsen, S.P. Jonstrup, L. Bigarré, M. Baud, T. Gray, P.M. Agapow and N.J. Olesen.** Fishpathogens.eu/noda : a free and handy online platform for Betanodavirus targeted research and data sharing. 16th International Conference on Diseases of Fish and Shellfish September 2 - 6, 2013, Tampere, Finland

Workshops:
1. MOLTRAQ, Berlin, May 19-23, 2014.
2. Molecular tracing of viral diseases in aquaculture. UM2, Montpellier, January 26-30, 2015
3. Molecular tracing of viral diseases in aquaculture. The 19th EAFP Conference, Canary Islands, Spain September 2015

Website:
www.moltraq.wordpress.com

Database:
www.fishpathogens.eu

Patents:
Not relevant for this project.

Summary of Work Packages

Project acronym: MOLTRAQ

Project full title: Molecular Tracing of viral pathogens in Aquaculture

Funded by the

Project start date: 01.04.2012

Project end date: 31.03.2015

Summary of Work Packages

Work Package 1		Start date or starting event (month):									1
Work package title	**Project co-ordination and consortium management**										
Participant No	**NVI**	DTU-VET	ANSES	FLI	IFREMER	IRD	NR				
Person-months per participant:	**4**	1	1	1	1	1	0,5				

Objectives (max. 1500 characters):

To draw up a consortium agreement, and make sure it is agreed upon and signed by all project partners.
To organise kick-off meeting and consortium meetings. These meetings will be held at different partner institutions, and thus the partners will be responsible for local planning.
To coordinate and manage all aspects of the contract management, including synchronising each partners contracts with their national funding organisations.
To maintain communication with EMIDA CO.
To keep the project on course with respect to economy, timetable and deliverables.
To provide progress reports on scientific and administrative matters according to guidelines from EMIDA CO.
To act in response to unforeseen events, such as delays, difficulties etc., by facilitating discussion and agreement between partners, and by communicating changes and decisions to the EMIDA CO.

Milestones (max. 1500 characters)

Please indicate Milestone number, - title and deadline (means month of project running time)
M1.1 Kick-off meeting to clarify interdependencies in the project and between partners, and to agree on detailed project planning. **Month 1**
M1.2 First progress meeting. **Month 7**
M1.3 Second progress meeting. **Month 22**
M1.4 Third progress meeting. **Month 34**

Deliverables (max. 1500 characters)

Please indicate Deliverable number, - title and deadline (means month of project running time)
D1.A Consortium agreement signed by all partners. **Month 1**
D1.B Mid-term report delivered. **Month 21**
D1.C Final report delivered. **Month 40**

Work Package 2		Start date or starting event (month):									1
Work package title	Collection of virus sequences and epidemiological data										
Participant No	NVI	DTU-VET	ANSES	FLI	**Ifremer**	IRD	NR				
Person-months per participant:	1	9	3	11	**7**	6	0				

Objectives (max. 1500 characters):

To assemble a complete inventory of strains, cultures, DNA, RNA and biological samples and associated biological and epidemiological information available among members of the consortium but also other research groups acting as strain external providers.
To investigate the genetic diversity of the collection using classic PCR-sequencing of appropriate genes/loci and select a sub sample of strains/cultures/DNA/RNA/biological samples for genomic approaches.
To provide material from all selected biological samples and perform genome sequencing.
To generate and collate all data necessary for conducting phylogenetic analysis and molecular epidemiological studies within the project.

Milestones (max. 1500 characters)

Please indicate Milestone number, - title and deadline (means month of project running time)
M2.1 Sample collections. Community samples collected and ready to use for the group. **Month 10**
M2.2 Sequencing of genomes from our collection of samples. Sequences available. **Month 20**
M2.3 Collating and systematize epidemiological data. Data available for the group. **Month 20**

Deliverables (max. 1500 characters)

Please indicate Deliverable number, - title and deadline (means month of project running time)
D2.A Samples collected and diversity analysis. **Month 10**
D2.B Whole DNA/RNA sequence for virus isolates. **Month 20**
D2.C Epidemiologic data available for the group. **Month 20**

Work Package 3		Start date or starting event (month):									3
Work package title	Phylogeny and evolution of viruses										
Participant No	NVI	DTU-VET	**ANSES**	FLI	Ifremer	IRD	NR				
Person-months per participant:	1	9	**11**	11	6	4	0				

Objectives (max. 1500 characters):

To collate the sequences obtained in WP2 as well as nucleic acid data of interest already published in databanks (viruses of reference).
To identify accurately (species/lineage) all selected viruses by phylogenetic analysis according to conventional methods (Neighbor-Joining, Maximum Parsimony, MLVA, etc.).
To determine genetic regions of particular interest (highly variable, linked to virulence) and study their mode of evolution (deletions / insertions, punctual mutations).
To choose representative genotypes for expression analysis in WP4.
To correlate the genetic, geographic and biological properties of the isolates and to emit preliminary hypotheses on the evolution of the studied viruses and their circulation before deeper analysis is performed in WP5.

Milestones (max. 1500 characters)

Please indicate Milestone number, - title and deadline (means month of project running time)
M3.1. Nucleic acid alignments and phylogenetic trees available for partners. **Month 21**
M3.2. Accurate genetic classification of viral isolates. **Month 24**

Deliverables (max. 1500 characters)

Please indicate Deliverable number, - title and deadline (means month of project running time)
D3.A. Taxonomy of the studied viruses based on phylogenetic analyses. **Month 20**
D3.B. Selection of genotypes of interest for expression analysis in WP4. **Month 21**
D3.C. Description of the modes of genetic variation for each model. **Month 24**
D3.D. Two publications dealing with genetic diversity and evolution in peer-reviewed journals, one for a RNA virus and one a DNA virus model **Month 36**

Work Package 4	**Start date or starting event (month):**										15
Work package title	**Investigation of the effect of temperature on gene expression patterns through DNA microarrays and real-time PCR**										
Participant No	NVI	DTU-VET	ANSES	FLI	Ifremer	**IRD**	NR				
Person-months per participant:	0	3	0	4	5	**21**	0				

Objectives (max. 1500 characters):

To develop a suitable microarray-based tool and real-time PCR assays for the identification of molecular markers responsible for virulence and/or pathogen spread.
To validate these tools for each investigated virus.
To select viral isolates to investigate according to their phenotype, genotype or genogroup.
To screen the transcription profile of the selected strains under different temperature conditions.

Milestones (max. 1500 characters)

Please indicate Milestone number, - title and deadline (means month of project running time)
M4.1. Design of the oligonucleotides. **Month 17**
M4.2. Validation of the oligonucleotide arrays. **Month 20**
M4.3. Identification of molecular markers involved in virus spread or virulence. **Month 26**
M4.4. Confirmation of expression profiles by real-time PCR. **Month 32**

Deliverables (max. 1500 characters)

Please indicate Deliverable number, - title and deadline (means month of project running time)
D4.A. Set of probes for each investigated viral pathogen. **Month 17**
D4.B. Molecular markers involved in the spread of viruses. **Month 32**
D4.C. Publications in peer-review journals on the molecular factors involved in virulence and/or virus spread. **Month 40**

Work Package 5							Start date or starting event (month):				1
Work package title	**Scenario simulation models for control options**										
Participant No	**NVI**	DTU-VET	ANSES	FLI	Ifremer	IRD	NR				
Person-months per participant:	**9**	2	0	1	1	2	6				

Objectives (max. 1500 characters):

To assemble complete spatio-temporal datasets representing different host-pathogen systems from the consortium members.
To analyse and parameterise stochastic spatio-temporal models for the spread of pathogens within the different consortium member host-pathogen systems.
To develop generic simulation modelling tools designed to explore effects of intervention strategies on the incidence of disease outbreaks in aquaculture systems.
To adapt simulation models to specific consortium member host-pathogen systems, and analyse these with respect to effects of intervention strategies on the incidence of disease outbreaks.

Milestones (max. 1500 characters)

Please indicate Milestone number, - title and deadline (means month of project running time)
M5.1. Completion of spatio-temporal data sets. **Month 20.**
M5.2. Parameterisation of stochastic models. **Month 25.**
M5.3. Development of generic simulation modelling tools. **Month 25.**
M5.4. Analyses of intervention effects for different host – pathogen systems. **Month 36**.

Deliverables (max. 1500 characters)

Please indicate Deliverable number, - title and deadline (means month of project running time)
D5.A. Software for generic simulation modelling made available. **Month 25.**
D5.B. Two or more publications in peer-review journals on intervention effects in different host – pathogen systems. **Month 36.**

Work Package 6		Start date or starting event (month):									1
Work package title	Dissemination and exploitation										
Participant No	NVI	DTU-VET	ANSES	FLI	Ifremer	IRD	NR				
Person-months per participant:	3	**6**	3	2	1	3	1,5				

Objectives (max. 1500 characters):

To publicise the aims and objectives of the project to the public, potential stakeholders and the scientific community- e.g. through establishment of a web site.
To expose the results obtained in the project at scientific conferences and meetings.
To present the results from the evaluation of strategies for disease surveillance and control to stakeholders.
To organise a workshop for stakeholders and the public and scientific community regarding the results on molecular tracing of viral pathogens, e.g. back to back with Annual Meetings of the EU Reference Laboratories for fish and molluscs, respectively.
To organise training courses for post-docs, Phd students and technicians involved in the project, e.g. course in micro-array technology for aquatic animal diseases.
To initiate patenting for any inventions, if applicable.
To publish research outcome in scientific peer reviewed journals.
To communicate main findings of an applicable nature to industry in non-scientific publications and meetings.

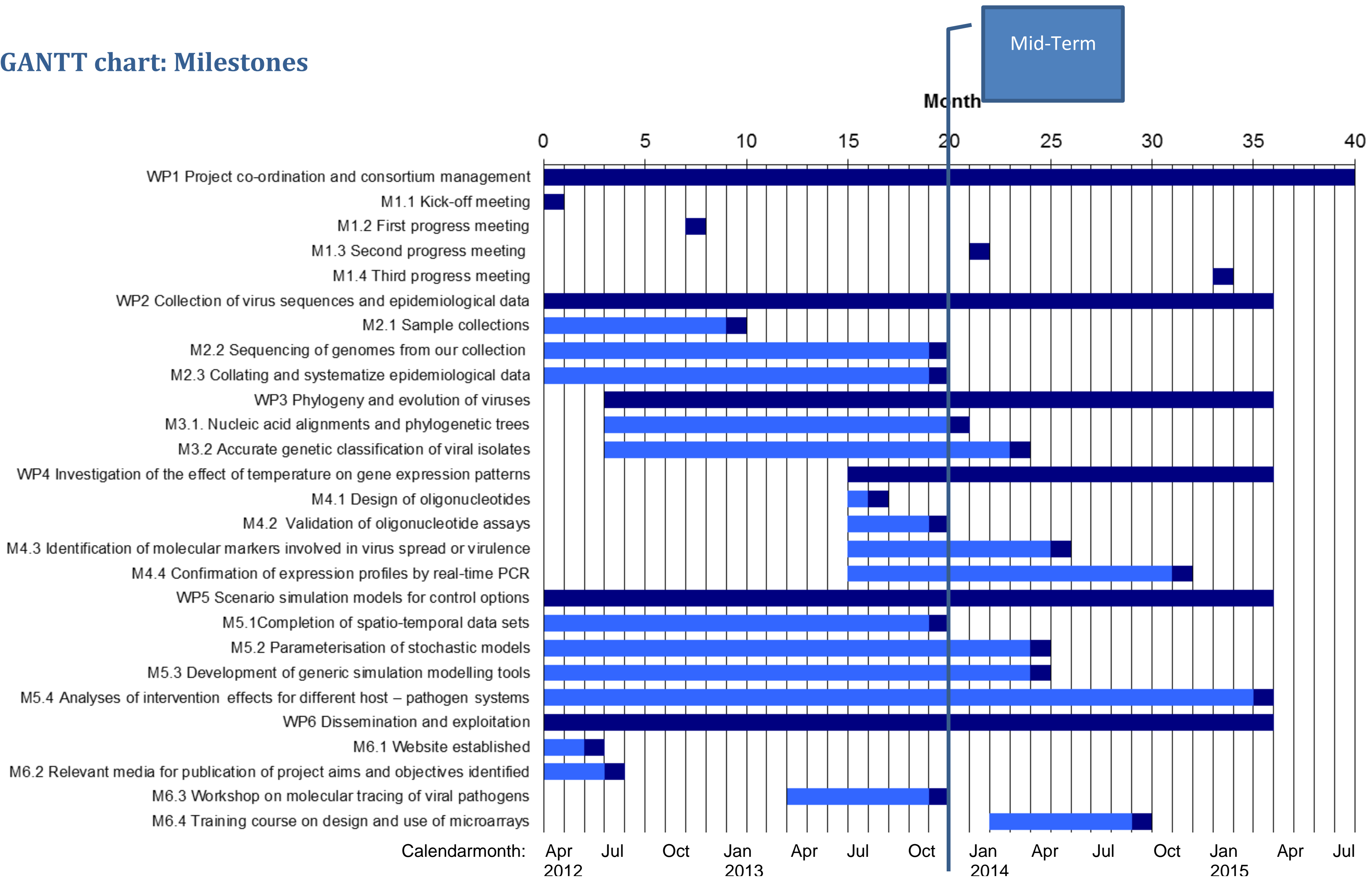
Milestones (max. 1500 characters)

Please indicate Milestone number, - title and deadline (means month of project running time)
M6.1. Web site established. **Month 3**
M6.2. Relevant media for publication of project aims and objectives identified. **Month 4**
M6.3. Workshop or course on molecular tracing of viral pathogens conducted and reported. **Month 20**
M6.4. Training course on design and use of microarrays **Month 30**

Deliverables (max. 1500 characters)

Please indicate Deliverable number, - title and deadline (means month of project running time)
D6.A. A web site established giving summary and description of the project with links to relevant databases. **Month 3**
D6.B. Scientific publication plan for all participants involved. **Month 12**
D6.C. Workshop on molecular tracing organised and held. **Month 20**
D6.D. Training course on design and use of microarrays for aquatic animal diseases. **Month 30**
D6.E. Final Report on project disseminations. **Month 40**

GANTT chart: Milestones



GANTT chart: Deliverables