VETBIONET

Veterinary Biocontained facility Network for excellence in animal infectiology research and experimentation

Deliverable D8.2

Development of alternative ELISAs for fish disease diagnostics and surveillance

Due date of deliverable: M48

Actual submission date: M72

Start date of the project: March 1st, 2017 **Duration:** 72 months

Organisation name of lead contractor: FLI

Revision: V1

Dissemination level	
Public	**X**
Confidential, only for members of the consortium (including Commission Services)	
Classified, as referred to in Commission Decision 2001/844/EC	

Table of contents

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

1. Summary

Deliverable D8.2 “Development of alternative ELISAs for fish disease diagnostics and surveillance” describes the Joint Research Activities (JRAs) performed and results obtained by the participating partners in WP8 Task 8.5.

Objectives:

The objectives of the JRAs in Task 8.5 were:

1. The production of retroviral vectors and stably IgT expressing rat cells (FLI)

2. The production, screening and characterization of rat mAbs against IgT (FLI/INIA/MS)

3. The establishment of IgT-ELISAs using full virus or recombinant antigen and archived sera from ISAV- (MS), VHSV- (FLI & INIA), and SAV-infected fish (MS) (in link to WP7)

4. The utilization of newly developed ELISAs for clinical samples taken at different time points after infection

Rationale:

The workflow was planned to consist in:

1. Development of monoclonal antibodies against Atlantic salmon and rainbow trout IgT

2. Establishment of ELISAs suitable for the detection of Infectious Salmon Anemia Virus (ISAV), Viral Hemorrhagic Septicemia Virus (VHSV) and Salmonid Alphavirus (SAV)specific IgT in trout mucus and serum.

3. Infection of rainbow trout and Atlantic salmon with ISAV, VHSV and SAV to study the IgT responses in ISA, VHS and PD, respectively.

4. Drawing conclusions on disease surveillance in aquaculture.

Teams involved: FLI, INIA, MS (has left the consortium in 2019), IZSVe, VAL

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement N°731014

2. Introduction

Fish express three classes of antibodies: IgM, IgT, IgD. The most abundant class is IgM and all ELISAs developed so far in any fish species are based on IgM. Since IgT seems to play an important role in fish immune responses, especially in the mucosal organs, our goal was to develop a corresponding IgT-ELISA. Furthermore, protection against certain fish diseases does not always correlate with the IgM levels in sera of immunized/infected fish. There is no information what the role of IgT in disease is and if the level of IgT in serum and mucus correlates with protection in certain fish diseases.

3. Results

VAL has identified and aligned several salmonid IgT isoforms to identify conserved regions. Synthetic peptides were used to develop two monoclonal antibodies (mAbs) VBN1 and VBN2. A sandwich ELISA to measure total IgT was developed, and this sandwich ELISA gave potentially positive data using VBN2 to capture and biotinylated VBN1 for detection which was in agreement with work at FLI were another anti-IgT-antibody from a third-party investigator was applied. Using mAbs VBN1 and VBN2, promising results with IgT ELISA using sera from trout infected with *Tetracapsuloides bryosalmonae* (causative agent of PKD) were obtained at VAL.

INIA produced a recombinant 200 aa segment of the CH region of rainbow trout IgT. The protein was used to immunize mice and to obtain a monoclonal antibody against the three IgT isoforms. In principle, the CH region was quite conserved among the three IgT isoforms. A positive hybridoma clone was identified. The resulting mAb efficiently recognized IgT in trout sera through Western blot and did not cross-react with IgM or IgD. The mAb also efficiently worked in immuno-fluorescence and -histochemistry, but turned out of being not suitable for use in an IgT ELISA.

FLI has developed rat cell lines stably expressing IgT that were injected into rats of similar genetic background to produce mAbs. However, no suitable clones could be isolated from rat spleens. Using another anti-IgT mAb from a third-party source as well as the VBN1 and VBN2 mAbs from VAL several IgT ELISA modifications were developed using sera from SAV- and PKD-infected fish. The general problem in developing IgT ELISAs is the low ratio of IgT when compared to IgM resulting in a sterical competition where IgM outcompetes IgT molecules so that IgT cannot be detected by anti-IgT mAbs during the ELISA protocol. Due to the issue with abundant/competing IgM we have developed different protocols (magnetic depletion, absorption) to deplete IgM from fish sera before using these sera in IgT ELISA. Magnetic beads

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

turned out to be most suitable for effective removal of IgM from sera of PKD and SAV infected trout. Positive antigen-specific IgT signals could be recorded in PKD-ELISA using a recombinant protein from *Tetracapsuloides bryosalmonae*, but at rather low levels and with some background signals which seem to be unique to PKD sera.

IZSVe's duties in this WP was to provide sera that potentially include IgT antibodies. However, since no feasible ELISAs could be provided these sera have not been tested so far.

4. Conclusions

An experimental-stage IgT ELISA along with mAbs against IgT was developed under Task 8.5. More efforts, time and resources are needed to establish a practically relevant IgT ELISA that can be used under the conditions of a diagnostic lab.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014