VETBIONET

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

Deliverable D8.5

Utilisation of NGS based tools to explore the T and B cell repertoires in domestic livestock before and after infection with BSL3 pathogens

Due date of deliverable: M60

Actual submission date: M72

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

Organisation name of lead contractor: MRI

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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

D8.5: *Utilisation of next generation sequencing (NGS) based tools to explore the T and B cell receptor repertoires in domestic livestock before and after infection with BSL3 pathogens*. In livestock species, sequencing of the highly variable regions of T cell receptor (TCR) and B cell receptor (BCR) transcripts provide a repertoire and frequency of variable gene usage, which improves our understanding of the adaptive immune response to infection or vaccination and will contribute to the rational development of new vaccines and immune-based therapies.

Objectives: The overall objective of WP8 is to deliver novel analytical tools and techniques, which will improve the capability of high containment laboratories, and ultimately the control and eradication of important pathogens of livestock. The WP8 technical objective associated with D8.5 is to provide advances in the development of genomics and bioinformatics tools targeting the adaptive immune response and focusing on the antigen receptor repertoire of T cells and B cells. This will be achieved through the development of high throughput genotyping technologies targeting T cell receptor (TCR) and the B cell receptor (BCR) repertoires in livestock populations undergoing infection, vaccination, or model development.

Rationale: D8.5 is associated with Task 8.2 and MS37 which focuses on the development of novel high throughput NGS sequencing and associated bioinformatics platforms to enable high resolution of the TCR-B gene repertoire analysis for cattle, and MS38, which applies this technology to samples to groups of pathogen infected cattle. This will enable T cell clonal expansion to begin to be defined ex vivo. Verification of completion of MS37 and MS38 (TCR-B) is through publication.

D8.5 is also associated with MS39 which focuses on the development of NGS sequencing and bioinformatics platforms to enable high resolution analysis of the highly variable CDR3 region of the BCR repertoire in cattle, and pigs. Once developed, these platforms will be applied to B cell samples isolated from cattle and pigs after infection with a BSL3 pathogen to monitor B cell expansion ex vivo. Verification of completion of MS39 is through publication.

Teams involved:

RI, ANSES, TPI, INRAE, AU, MRI

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2. Introduction

A central feature of the immune response is the ability to recognize and respond to initial encounter with a pathogen or vaccine and to respond in a quicker and more targeted way on subsequent pathogen encounter. This immune memory provides the basis for vaccination. This is achieved through adaptive T cell and B cell responses and involves host MHC proteins which present small peptides derived from a pathogen or vaccine preparation to antigen specific T cell receptors expressed by immunoregulatory T lymphocyte populations. These T cells rapidly expand and co-operate with both cytotoxic T cells, which kill pathogen infected cells and B cells activated through their antigen specific B cell receptors to produce antigen specific immunoglobulin. While deliverable 8.4 focused on the development of sequence-based tools to analyze diversity in the MHC genes, deliverable 8.5 focuses on developing sequence-based tools to rapidly analysis diversity in the T cell receptor (TCR) and B cell receptors (BCR) repertoires in livestock populations responding to infection.

3. Results

3.1 Result 1: The development of high throughput TCR genotyping technologies for cattle

Work package co-ordination: Work package co-ordination activities were progressed in the initial phase of the project through face to face and virtual meetings involving all partners. Minutes were circulated and forwarded to WP lead and VetBioNet coordinator. These meetings continued ad hoc during Covid lockdowns when face to face meetings were not possible. Importantly, technology exchange, method optimization and primer sequence information flowed between partners. A training workshop in this area was planned to be hosted at RI which had to be cancelled due to Covid-19 lockdowns.

Method development and technology transfer: PCR primers, each with unique molecular barcodes, were designed allowing multiplexing of amplicons targeting the CDR3 domain of the cattle TCR B gene. Amplicons generated by RT-PCR from different animals were pooled and libraries sequenced on the Illumina MySeq platform. A prototype bioinformatic pipeline for sequence analysis was designed and established at RI. The bioinformatic pipeline was optimised, and analysis of sequence data completed at RI (MS37 and 38). The method and bioinformatic pipeline for bovine TCR repertoire analysis using MiSeq has been shared with project partners in ANSES and EU collaborators for analysis of T cell responses to Malignant Catarrhal Fever (MCF) in cattle (Benjamin Dewals - Uni. of Liege, Belgium). The method has recently been applied to the analysis of T cell responses to the zoonotic bacterial pathogen of cattle, *Leptospira* and the details reported in the publication below.

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Milestone 37 and 38 verification: Publication of validated method:

γδ TCRs Function as Innate-like Receptors in the Bovine γδ T Cell Response against *Leptospira* Gillespie A.E, Loonie K, Lefevre, Hope J.C, Baldwin C.L, Connelley T.C. J Immunol October 7, 2022, ji2200319; DOI: https://doi.org/10.4049/jimmunol.2200319.

Oral presentations (in French)

Développements méthodologiques au service de la vaccinologie porcine [Methodological developements at the service of porcine vaccinology] - *Réunion d'information et d'échange de l'Anses - Secteur porcin*, *Ploufragan - 30 novembre 2018*

Analyse des répertoires en anticorps chez le porc, le poulet et la truite par séquençage haut-débit [Analysis of antibody repertoires in pigs, chickens and trout by high-throughput sequencing] - *Journées Scientifiques et Doctorales de l'Anses - Maisons-Alfort - 5 et 6 décembre 2018*

3.2 Result 2: The development of high throughput BCR genotyping technologies for Swine Chicken and Fish

Initial experiments to amplify the porcine B-cell receptor CDR3 region were completed. Primer pairs were designed for RT-PCR using RNA samples extracted from porcine peripheral blood mononuclear cells (PBMC) and the amplicons sequenced on the Ion Torrent NGS platform. The variable CDR3 regions were analyzed using V-Quest and High V-Quest bioinformatic tools. Initial data analysis provided promising results as the CDR3 region was identified in many of the amplicon sequences. The bioinformatic pipeline for analysis of NGS sequence data has been improved and a workflow developed for sequencing the CDR3 region of the porcine BCR repertoire for studies in which samples are prepared at various time points before and after infection or vaccination.

The workflow has been applied to the analysis of the B-cell receptor CDR3 repertoire in pigs infected with a range of pathogens including Porcine Epidemic Diarrhea Virus, Swine Influenza Virus and Salmonella. Other samples from pigs immunized with a prototype vaccine against

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African Swine Fever Virus were discarded as the vaccine prototype failed to induce an antibody response.

In the initial optimization studies, different sources of RNA were compared (whole blood, purified PBMCs and RNA extracted from whole blood using the leukoLOCK system). Two high throughput sequencing platforms were compared: Ion Torrent and Illumina MySeq. IMGT High V-Quest and IMGTStatClonotype software platforms were used to analyze the sequences obtained from both sequencing platforms.

Both sequencing platforms generated BCR CDR3 sequence and more sequences were obtained from RNA extracted from purified PBMCs. In each case, it was possible to identify the antibody isotype associated with the BCR CDR3 sequences (IgA, IgM, IgG, IgD and IgE). Furthermore, it was possible to identify changes in the BCR CDR3 repertoires at time points before and after infection (CDR3 length, VDJ usages, CDR3 sequences). No common BCR CDR3 sequences (public antibody specificities) were identified in different individuals infected with the same pathogen.

A second study used RNA derived from PBMC isolated from four pigs immunized against porcine atypical pestivirus before immunization and seven days post vaccine boost. The Illumina MySeq platform was used for sequencing the BCR CDR3 repertoire and Vidjil software was used to analyze the sequence data. Here, differences in the repertoire of BCR CDR3 were identified before and after immunization. However, in this study a common CDR3 sequence (public CDR3) was identified in two of the immunized pigs. These results must be validated by extending the analysis to samples at 21 days post-immunization.

The third set of studies aimed to determine the B-cell CDR3 repertoire in six pigs before and eight days after infection with swine influenza virus and six pigs before and after infection with PRRSV. These studies were delayed due to the Covid pandemic and subsequent staffing issues. However, the 24 sequencing libraries from these studies have recently been sequenced and data analysis is ongoing.

Material was prepared and archived for development of chicken B-cell CDR3 repertoire analysis. This included bursa of Fabricius and spleen tissues collected from vaccinated and unvaccinated Ross and Leghorn chickens infected with Campylobacter at 0, 3, 9, 16 and 23 days post infection. A range of PCR primers sets were identified. However, these studies were not progressed further as we became aware that identical technology was being developed in

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Chickens in a laboratory independent of VetBioNet. The decision was therefore made to focus work package resources on the swine BCR repertoire work.

Rainbow Trout BCR work was initiated using spleen samples from trout infected with infectious hematopoietic necrosis virus. RNA was isolated from trout infected with a highly virulent IHNv strain at 8 weeks post infection and a contemporary non-infected trout. However, despite generating a range of PCR primers sets, we were unable to generate sufficient numbers of Trout BCR sequences to compare viral infected and control samples. As such, it was decided to concentrate resources on the swine BCR work.

Milestone 39 *TRB sequences analysis validated using in vivo samples from pigs, chickens, and fish.* Means of verification: Publication of validated method:

Due to the preliminary nature of the methodology development, no publications have been prepared.

4. Conclusions

In conclusion, we have succeeded in developing and applying NGS sequence-based technologies for analysis of TCR repertoire in cattle (MS36 and MS37). The method and bioinformatics platform has been submitted, which on publication provides verification of MS36 and MS37. The technology has been exchanged between project partners and with European collaborators interested in TCR repertoire analysis challenge. This technology increases the range of tools available for analysis of immune responses in cattle undergoing high containment infection and vaccination studies.

The development of related technology for analysis of the BCR repertoires in swine, chicken and fish has proved to be more challenging. Archives of samples from swine, chicken and trout infection as well as vaccination studies were generated and nucleic acid prepared. Using samples from pathogen infection and vaccination studies, significant success has been achieved for the swine BCR repertoire analysis and data analysis is ongoing. Technical delays and the Covid pandemic have limited progress with BCR repertoire technology development in chicken and fish.

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