PhD PROPOSAL FOR THE DOCTORAL SCHOOL
« Ecologie, Géosciences, Agronomie, ALimentation »

GENERAL INFORMATION

Thesis title: Sequencing of porcine antibody repertoires: identification of CDR3 marker sequences and obtaining complete antibody sequences. Application to porcine respiratory complex viruses and porcine pestiviruses.

Acronym: SeqAPorc

Disciplinary field 1: Food sciences

Disciplinary field 2: Select an element

Three keywords: antibodies – diversity – viral infections

Research unit : Viral Genetics and Biosafety Unit, ANSES (FRENCH AGENCY FOR FOOD, ENVIRONMENTAL AND OCCUPATIONAL HEALTH & SAFETY) Laboratory of Ploufragan-Plouzane-Niort

Name of the thesis director HDR (Habilitation thesis to supervise research) required: Daniel DORY

Email address of the thesis director: daniel.dory@anses.fr

Name of the thesis co-director (if applicable): HDR (Habilitation thesis to supervise research) required:

Email address of the thesis co-director (if applicable): John Hammond

Name of the thesis co-supervisor 1 (if applicable): john.hammond@pirbright.ac.uk

Email address of the thesis co-supervisor 1 (if applicable):

Name of the thesis co-supervisor 2 (if applicable):

Email address of the thesis co-supervisor 2 (if applicable):

Thesis grant (funding origin and amount): ANSES (48 k€) – Saint-Brieuc Armor Agglomération (24 k€) – Conseil Départemental des Côtes d'Armor (24 k€)

Contact(s) (mailing address and E-mail): ANSES, 41 rue de Beaucemaine, 22440 Ploufragan – daniel.dory@anses.fr

SCIENTIFIC DESCRIPTION OF THE PhD PROJECT

Socio-economic and scientific context : (10 lines)

There are more than 1100 pig farms, large slaughterhouses and cooperatives or groups of breeders in Brittany. Pork meat production is therefore one of the pillars of the local economy which directly and indirectly supports thousands of people. The health issues of pig farms are varied and range from animal welfare to the prevention of economic risks associated with animal diseases. To preserve animal health, it is essential to acquire new tools for both the diagnosis of infectious diseases and the identification of new potential vaccines. Here, the strategy is based on the analysis of pig antibody repertoires. These repertoires indeed reflect the infectious background of animals as well as the immune response of the animal developed to fight these pathogens. Moreover, the technology that will be developed here is a universal technology potentially applicable to other livestock species (e.g., cattle and poultry).

Assumptions and questions (8 lines)

The antibody repertoire represents all the antibodies coded by an individual against immunogenic epitopes of various origins: infectious, vaccinal or allergic. The set of antibodies therefore depends on encountered immunogens and reflects the individual's natural protective response to pathogens. Pig antibodies maturation is highly dependent on high rates of somatic hypermutation, key to the antibody repertoire diversification, which should be specifically target for analysis with specialized tools. The analysis of antibody repertoires in infected pigs with specific viruses of interest at ANSES should make it possible to (1) have new diagnostic tools (antibody signatures) and (2) serve as a basis for the development of new vaccines.

The main steps of the thesis and scientific procedure (10-12 lines)

1. Collection and preparation of porcine antibody repertoire samples

Pig blood samples will be used to amplify and sequence antibody repertoires from circulating B cells. Those will be from pigs infected with a porcine respiratory complex virus or pestiviruses. RNA will be extracted from PBMCs and frozen until further processing.

2. High-throughput sequencing and analysis of porcine B repertoires from different samples

Antibodies' heavy and light chains will be amplified and indexed by PCR followed by library preparation and next generation sequencing. Antibody sequences will then be analyzed using specific bioinformatic pipelines. Initially, the analysis will only focus on the most variable region of each antibody, the CDR3. A portion of these antibodies have been mutated and selected to be as effective as possible in the fight against the pathogen they encountered. Since antibodies are the product of gene segments recombination and somatic hypermutation, it is essential to use specific antibody sequence analysis tools that consider all these phenomena, in particular to search for shared antibodies between individuals (so-called public antibody sequences).

3. Complete sequencing of antibodies of interest and production of recombinant antibodies

Antibodies are composed of a light and a heavy chains, which need to be accurately paired to recapitulate the antibody specificity and avidity. Carrying out "single cell" work on one or a few samples which were of interest in stage 2, will enable to sequence antibody natural chains pairs, which can further be used to generate a recombinant antibody. Expression of the recombinant antibodies will enable to characterize them, and define the antigens they recognize. This will open up new perspectives for the development of new vaccines, based on the natural response of animals to the pathogens of interest.

Methodological and technical approaches considered (4-6 lines)

- Blood samples processing for the analysis of antibody repertoires: isolation of PBMCs, in vitro stimulation, cell lysis and RNA extraction.
- RT-PCR, library preparation for NGS sequencing
- Interactions with the NGS platform and bioinformaticians
- Bioinformatics analysis of antibody repertoires

- Preparation of cells for single cell transcriptomic analysis
- Production of a recombinant antibodies

Scientific and technical skills required by the candidate
The candidate should be familiar with or be interested in immunology techniques, in particular the isolation and culture of lymphocytes, molecular biology techniques (e.g. extraction of RNA or DNA from lymphocytes, PCR, nucleic acid sequencing). They will also collaborate with the high-throughput sequencing team and the bioinformatics team. They must also have knowledge of virology. Finally, fluency in English is important to communicate efficiently with Pirbright's co-supervisor and team. They will be required to travel punctually to the United Kingdom.

THESIS SUPERVISION[1]

Unit name:	**Team name:**
ANSES Laboratory of Ploufragan – Plouzané - Niort	Viral Genetics and Biosafety Unit
Unit director name:	**Team director name:**
Nicolas Eterradossi	Yannick Blanchard
Mailing address of the unit director:	**Mailing address of the team director:**
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Thesis director
Surname, first name: DORY Daniel
Position: Scientist
Obtained date of the HDR (Habilitation thesis to supervise research): February 2nd 2010
Employer: ANSES
Doctoral school affiliation: EGAAL « Écologie, Géosciences, Agronomie, ALimentation », Rennes, France
Rate of thesis supervision in the present project (%): 50
Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%): 100
Number of current thesis supervisions/co-supervisions: 2
Thesis co-director
Surname, first name:

Position:

Obtained date of the HDR (Habilitation thesis to supervise research):

Employer:

Doctoral school affiliation:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

Thesis co-supervisor 1 (if applicable)

Surname, first name:

Position:

Habilitation thesis to supervise research ☐ yes ☐ no   If yes, date diploma received:

Employer:

Doctoral school affiliation:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

Thesis co-supervisor 2 (if applicable)

Surname, first name:

Position:

Habilitation thesis to supervise research ☐ yes ☐ no   If yes, date diploma received:

Employer:

Doctoral school affiliation:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

Private partner (if CIFRE funding, private funding,…)

Surname, first name:

Position:

Employer:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

International partner (if Cotutelle thesis)

Surname, first name: Hammond, John

Position: Professor

Employer: Pirbright Institute UK

Rate of thesis supervision in the present project (%): 50

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

Professional status of previous PhD students supervised by both director and co-supervisors (from 5 years)

Please provide the following information for each PhD students supervised

Surname, first name: Meunier Marine

Date of PhD beginning and PhD defence: 01/03/2014 – 24/04/2017

Thesis supervision: Marianne Chemaly 50 % (HDR) et Daniel Dory 50 % (HDR)

Professional status and location: Scientist, pharmaceutical industry

Contract profile (post-doc, fixed-term, permanent): permanent

List of publications from the thesis work:
1. M. Meunier, M. Guyard-Nicodème, E. Vigouroux, T. Poezevara, V. Béven, S. Quesne, M. Amelot, A. Parra, M. Chemaly, D. Dory. A DNA prime/protein boost vaccine protocol developed against Campylobacter jejuni for poultry. Vaccine, 2018, 36, 2119-25
2. M. Meunier, M. Guyard-Nicodème, E. Vigouroux, T. Poezevara, V. Beven, S. Quesne, L. Bigault, M. Amelot, D. Dory, M. Chemaly. Promising new vaccine candidates against Campylobacter in broilers. PlosONE, 2017, 12, e0188472
3. M. Meunier, M. Guyard-Nicodème, E. Hirchaud, A. Parra, M. Chemaly, D. Dory. Identification of Novel Vaccine Candidates against Campylobacter through Reverse Vaccinology. Journal of Immunology Research 2016, 2016, 5715790
4. M. Meunier, M. Chemaly, D. Dory. DNA vaccination of poultry: The current status in 2015. Vaccine. 2016, 34, 202-11
5. M. Meunier, M. Guyard-Nicodème, D. Dory, M. Chemaly. Control Strategies against Campylobacter at the Poultry Production Level: Biosecurity Measures, Feed Additives and Vaccination. Journal of Applied Microbiology. 2016, 120, 1139-73

Surname, first name: SOUCI Laurent

Date of PhD beginning and PhD defence: 01/01/2017 – 30/11-2019 / defense 9/12/2020

Thesis supervision: Daniel Dory 100 % (HDR)

Professional status and location: Ingeneer, INRAE, Tours, France

Contract profile (post-doc, fixed-term, permanent): permanent

List of publications from the thesis work:
L. Souci, H. Jaunet, G. Le Diguerher, J.M. Guionnet, V. Béven, F. Paboeuf, T. Montier, D. Dory. Intranasal inoculations of naked or PLGA-PEI nanovectored DNA vaccine induce systemic and mucosal antibodies in pigs: A feasibility study. Research in Veterinary Science 2020, 132:194-201.

Surname, first name: Le Du-Carré Jessy

Date of PhD beginning and PhD defence: 01/01/2018 – 10/03/2021

Thesis supervision: Daniel Dory (HDR), Thierry Morin, Morgane Danion

Professional status and location: -

Contract profile (post-doc, fixed-term, permanent): -

List of publications from the thesis work:
Le Du-Carrée J., Morin T., Danion M. Impact of chronic exposure of rainbow trout, *Oncorhynchus mykiss*, to low doses of glyphosate or glyphosate-based herbicides. Aquatic Toxicology 2021, 230:105687.

Five main recent publications of the supervisors on thesis subject:
1. Dorey-Robinson D, Maccari G, Borne R, **<u>Hammond JA</u>**. 2021. IgMAT: immunoglobulin sequence multi-species annotation tool for any species including those with incomplete antibody annotation or unusual characteristics.
2. L. Souci, H. Jaunet, G. Le Diguerher, J.M. Guionnet, V. Béven, F. Paboeuf, T. Montier, **D. Dory***. Intranasal inoculations of naked or PLGA-PEI nanovectored DNA vaccine induce systemic and mucosal antibodies in pigs: A feasibility study. Research in Veterinary Science 2020, 132:194-201.
3. M. Meunier, M. Guyard-Nicodème, E. Vigouroux, T. Poezevara, V. Béven, S. Quesne, M. Amelot, A. Parra, M. Chemaly, **D. Dory***. A DNA prime/protein boost vaccine protocol developed against Campylobacter jejuni for poultry. Vaccine, 2018, 36, 2119-25
4. M. Meunier, M. Guyard-Nicodème, E. Vigouroux, T. Poezevara, V. Beven, S. Quesne, L. Bigault, M. Amelot, **D. Dory**, M. Chemaly*. Promising new vaccine candidates against Campylobacter in broilers. PlosONE, 2017, 12, e0188472
5. Holzer B, Rijal P, McNee A, Paudyal B, Martini V, Clark B, Manjegowda T, Salguero FJ, Bessell E, Schwartz JC, Moffat K, Pedrera M, Graham SP, Noble A, Bonnet-Di Placido M, La Ragione RM, Mwangi W, Beverley P, McCauley JW, Daniels RS, **<u>Hammond JA</u>**, Townsend AR, Tchilian E. 2021. Protective porcine influenza virus-specific monoclonal antibodies recognize similar haemagglutinin epitopes as humans. PLoS Pathog 17:e1009330.

THESIS FUNDING

Origin(s) of the thesis funding: ANSES – SAINT-BRIEUC ARMOR AGGLOMERATION – CONSEIL DEPARTEMENTAL 22

Gross monthly salary: ABOUT 1750 € BRUT

Thesis funding state : Acquired

Funding beginning date/Funding ending date: BETWEEN 1ST OCTOBER 2022 – 1ST JANUARY 2023 – 3 YEARS

DOCTORAT / **ECOLOGIE**
BRETAGNE \ **GEOSCIENCES**
LOIRE / **AGRONOMIE ALIMENTATION**

Date: 9 JULY 2022

Name, signature of unit director: ETERRADOSSI NICOLAS (has signed the French version)

Name, signature of team director: BLANCHARD YANNICK (has signed the French version)

Name, signature of thesis project director: DORY DANIEL