VetBioNet

Veterinary Biocontained facility Network for excellence in animal infectious disease research and experimentation

Newsletter – Issue 3

February 2021

The VetBioNet project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement N°731014

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Summary

VetBioNet Transnational Access opportunities

VetBioNet, "Veterinary Biocontained facility Network for excellence in animal infectious research and experimentation", is an infrastructure project funded by the European Union's Horizon 2020 Research and Innovation Program under grant agreement No 731014. The aim of VetBioNet is to strengthen the European capacity and competence to meet the challenges of epizootic and zoonotic diseases by establishing a durable network of Europe's leading bio-contained research infrastructures, international organisations and industries and by offering cutting-edge services (TNA).

Transnational Access (TNA)

VetBioNet offers free-of-charge access to the high-containment animal facilities, analytical platforms, sample collections and services of Europe's leading research infrastructures in animal infectious disease research. This access/service offer is directed towards academic or public research laboratories and private enterprises that are lacking the necessary infrastructure resources (especially bio-contained animal/laboratory facilities) to conduct research studies on high-risk epizootic and zoonotic diseases. Diseases that can be studied include **OIE-listed diseases** (e.g. African Swine Fever, Food and Mouth Disease, Peste de Petits Ruminants, West Nile Fever, Rift Valley Fever, bTB, Rabbit Haemorrhagic Disease, Avian

Influenza, Infectious haematopoietic necrosis, Spring Viraemia of Carp), **COVID-19, SARS, MERS** and certain non-OIE-listed **production diseases** in livestock and fish (e.g. Marek's Disease, Chicken erysipelas, Porcine Pleuropneumonia, Nervous Necrosis). Transnational Access (TNA)* can be granted to applicants proposing a sound project (basic research, R&D and preclinical studies) related to such epizootic and zoonotic disease risks.

Detailed information about the **straightforward TNA application procedure**, service providers and the online application form can be found on the VetBioNet website. Full project proposals do commonly not exceed five pages, and VetBioNet strives to make **swift funding decisions within a six-weeks' time frame**.

Please note: EU research infrastructure rules stipulate that, to benefit from a VetBioNet TNA grant, applicants (i) must not be part of the VetBioNet consortium and (ii) can only apply for services from (a) VetBioNet service provider(s) that is/are located outside the applicant's home country (transnational access rule).

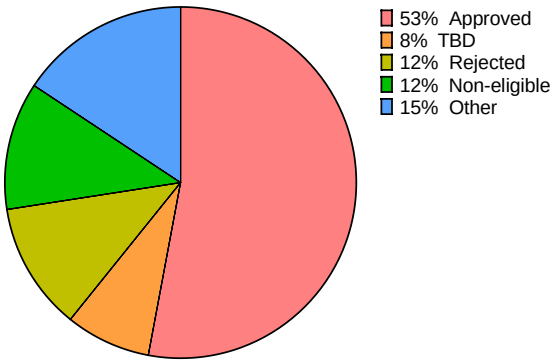
*Free-of-charge access to:

- **BSL-2/BSL-3 animal facilities**
  Livestock, ferrets, rodents, wildlife species and fish (incl. animal purchase and husbandry)
- **Animal study support**
  Inoculation, clinical monitoring, post-mortem, pathological scoring, sampling & sample processing
- **Laboratory capacities and expertise**
  Pathogen quantification, serological monitoring, histopathological scoring, data analysis etc.
- **State-of-the-art technical platforms**
  OMICS, imaging, cell sorting, telemetry etc.
- **Biobanks**
  Infectious or decontaminated serum and tissue samples, cell lysates, nucleic acids etc.
- **Sample on demand services**
  Customised preparation of non-infectious or decontaminated samples from BSL-3 experiments for downstream analyses in a conventional BSL-1 laboratory

Travel and accommodation expenses or sample shipment costs may also be included in the service package dependent on the VetBioNet service provider.

Please also consult the appended VetBioNet TNA Project Call flyer and feel free to share it with your professional network.

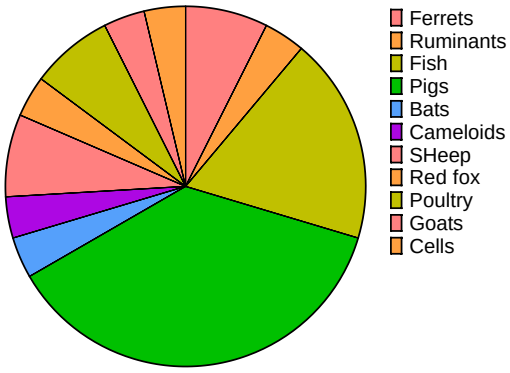
Overview: VetBioNet TNA project applications 2018-2020



Total=51

The VetBioNet TNA project call has been launched in 2018 and is now active for almost 3 years. During this period, 51 applicants have submitted a project proposal. Of these proposals, **53% were granted Transnational Access to the facilities and/or services of the VetBioNet service providers**. Twelve percent of the proposals were rejected, 12% were deemed not eligible (on the pre-proposal level) and 15% were not considered for other reasons (e.g. withdrawal by the applicant).

Until today, **27 applicants have benefitted from a grant** permitting them to perform studies in the bio-contained facilities and with the support of a VetBioNet service provider. The topics studied were relatively diverse: **19 different pathogens** were studied in **10 different animal species**.



Total=27

Due to the broad expertise available at the service provider organisations, **a large variety of animal species can be used** ranging from livestock animals to teleost fish as well as more 'exotic' animals (reservoir hosts) such as camelids and red foxes.

Completed VetBioNet TNA projects: Testimonials

The four TNA projects outlined below illustrate how VetBioNet can help researchers to realise studies that require access to rare and costly infrastructure resources. What is more, these projects have also laid the foundations for future collaborations and knowledge transfer between the TNA users and the VetBioNet service providers.

Many thanks to Ana Balseiro, Robert Söderlund, Sofie Barsøe and Gaetano Donofrio for outlining their projects and providing feedback on their TNA user experience!

Experimental infection of red foxes (Vulpes vulpes) with a naturally virulent Mycobacterium bovis strain

TNA user: Dr Ana Balseiro, University of Léon, Spain.
TNA service provider: Laboratory for rabies and wildlife, ANSES Nancy, France.

Performing necropsy during the study ©Anses

Ana Balseiro, from the University of Léon, was granted access to the ANSES Nancy Laboratory for Rabies and Wildlife to take advantage of their specialised BSL-3 animal facilities and expertise with wild carnivore infection models. Her study was performed from October 2019 until October 2020.

Mycobacterium bovis excretion in urine and faeces in naturally infected foxes has previously been observed, but no experimental data were available on the pathogenesis of M. bovis infection in foxes and on the associated risk of excretion.

To validate an experimental infection protocol by the oral route, 12 captive foxes (6 females and 6 males) were infected with a M. bovis field isolate ($1.5x10^7$ CFU). Immunology, bacteriology and pathology protocols aligned with those already used with M. bovis experimentally infected European badgers and ferrets were performed 12 weeks post-infection. At post-mortem, only few

The other main members of the study (from left to right: Javier Salguero – PHE, UK; Maria-Laura Boschiroli, Sylvie Henault, Jacques Barrat and Céline Richomme – Anses, France; Sandrine Lesellier, APHA, UK), taken in front of the Cat3 facilities. ©Anses

macroscopic lesions were observed. Histology showed small granulomas within the lymph nodes, tonsils, liver and lung from a small number of foxes, with the presence of scarce acid-fast bacilli. All 12 foxes had at least four PCR positive samples (out of the 23 tested), and 11 foxes had at least on culture positive sample. The culture negative fox was PCR positive in both retropharyngeal and the mesenteric lymph nodes, in line with the results of the other animals. M. bovis was detected by PCR in the bladder of 3 foxes at 82 days post-infection and, during the trial, in faeces of 9 foxes as well as in the oropharyngeal mucus of 3 individuals.

This oral infection protocol was able to reproduce the pattern of infection observed in foxes that are naturally infected by tuberculosis (TB) in the wild and the data underpin the risk of excretion of mycobacteria by TB-infected foxes.

Dr Ana Balseiro on her experience as TNA user:

"Our Vetbionet project has given us the opportunity to evaluate how Mycobacterium bovis affects foxes, a species that had not been studied so far in this regard.
For us, it has been an amazing challenge"

Assessing the role of acute phase protein and opsonin MBL in protective immune responses against erysipelas in chickens

TNA user: Dr Robert Söderlund, National Veterinary Institute in Uppsala, Sweden.
TNA service provider: Aarhus University, Department of Animal Science, Denmark.

Robert Söderlund, from the National Veterinary Institute in Uppsala, was granted access to the Department of Animal Science of Aarhus University to take advantage of their BSL-2 poultry facility and their scientific expertise. His study ran from July to September 2019.

Erysipelas is a disease caused by infection with a bacterium named Erysipelothrix rhusiopathiae (ER). This disease is a major problem in laying hens, especially in organic production. To enable development of sustainable control measures for this disease, e.g. through vaccination, it is important to have knowledge on mechanisms in the chicken immune system involved in the control of infection. In previous studies, the plasma protein called mannose binding lectin (MBL) was indicated to have a role in the early immune responses of chickens to ER infection. The present study was therefore carried out with the aim to try and elucidate the role of MBL in ER infection by using inbred chicken lines with genetically high or low levels of MBL in blood that are available at the Department of Animal Science, Aarhus University.

Dr. Robert Söderlund

Chickens were hatched and kept in biosafe facilities. They were infected with ER and immune responses were monitored for 3 weeks after infection. The immune responses observed revealed several notable parameters, such as increased numbers and activation of immune cells and increased levels of MBL in blood. However, no firm conclusions on the role of MBL in the early immune responses could be drawn.

Chicks from an inbred line

Interestingly, activation of "helper" and "killer" T cells by ERs was also observed, making this study the first to investigate the response of T cells to ER infection in chickens, and strongly suggesting the involvement of these immune cells during ER infection. As these cells are involved in “immune memory”, they are important for development of protective immunity e.g. after vaccination.

Thus, the project generated novel knowledge on the chicken immune responses to ER, which will be important for control of erysipelas in laying hens.

Dr. Tina Sørensen Dalgaard on her experience as TNA provider:

"It was a very positive experience providing access to our inbred chicken lines and animal facility. The rich dialogue with the TNA user and his colleagues at the Swedish National Veterinary Institute was effortless and the good communication strengthened the design of the proposed study encompassing shared scientific interests and making the most of our respective expertise"

Vaccination of Seabass against a lethal viral disease and characterization of protective immunity

TNA user: Sofie Barsøe, Institute for Aquatic Resources, Technical University of Denmark, Kongens Lyngby, Denmark.
TNA service provider: Experimental Fish facility (IZSVe), Padova, Italy.

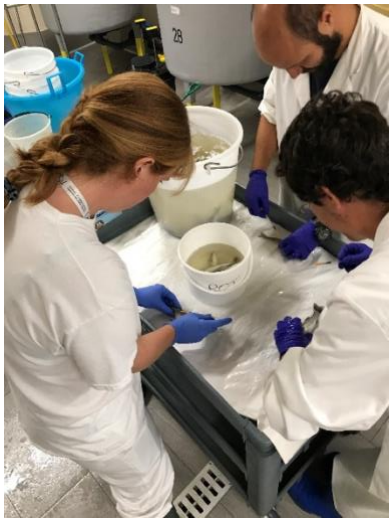
Tank facilities at IZSVe where the experiment was performed

PhD student Sofie Barsøe from the Technical University of Denmark, had the opportunity to visit the IZSVe, Experimental Fish facility in Padova, through the VetBioNet TNA programme. Her study was performed from July to September 2019.

During her stay at IZSVe, Sofie performed a vaccination and challenge study in European Sea Bass to test an experimental vaccine against two different strains of betanodavirus. Betanodavirus causes the disease Viral Nervous Necrosis (VNN) which is an infection of the CNS with serious neurological symptoms and high mortalities in marine species. The disease is a big problem in farms with sea bass, one of the main cultured species in the Mediterranean Sea. The work is part of Sofie's PhD at the Technical University of Denmark, where she is working on different aspects of the trial of a new experimental vaccine against VNN. As IZSVe is the OIE reference laboratory for VNN, this has been a unique opportunity for Sofie to visit IZSVe and to learn from their expertise.

Sofie Barsøe on her experience as TNA user:

"It was a very unique opportunity to be able to visit the Fish disease group at IZSVe and perform an experiment with NNV and sea bass. Because of the experiences and skills gained during my stay, but also because of the great basis for future collaboration and exchange of knowledge that has been established".



Virus inoculation of sea bass

Assessment of the immunogenicity of bovine herpesvirus 4-based vectors delivering Nipah virus glycoproteins in swine

TNA user: Prof Gaetano Donofrio University of Parma, Italy in collaboration with The Pirbright Institute, UK.
TNA service provider: Animal & Plant Health Agency (APHA), Weybridge, UK.

Gaetano Donofrio, senior researcher at the University of Parma, has been working for many years on the generation and the development of a bovine herpesvirus type 4 (BoHV-4)-based viral vector. The BoHV-4-based vector has great potential in the field of veterinary vaccinology, but development of such vaccines is often limited by the lack of biocontainment facilities for large animals where prototypes of BoHV-4 recombinant vaccines can be tested. This is further complicated for vaccines against zoonotic pathogens (that can spread from animals to humans) where a BSL-3 facility is required. Through VetBioNet TNA, Gaetano was granted access to the APHA large animal facilities in order to test two new potential vaccines against Nipah virus.

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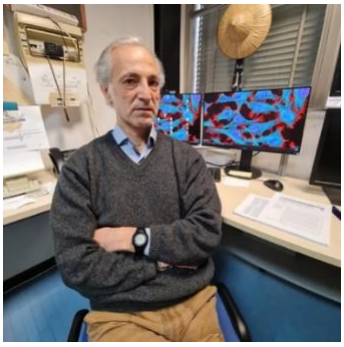
Nipah virus infection can cause respiratory problems and fatal encephalitis in pigs but also in humans. This virus typically infects bats, but pigs can become infected by consuming fruits contaminated with bat secretions. Spread of the virus from pigs to humans can also occur, as for the first and most severe human Nipah outbreak in Malaysia during 1998-99, where almost 200 pig

farmers died after contracting the virus from infected pigs. The eradication of Nipah from pigs in Malaysia was dangerous and costly (estimated US$582 million), requiring the army to cull almost half of the national herd.

To create these new vaccine candidates, BoHV-4 was genetically engineered to deliver Nipah virus G and F proteins to pigs. These proteins, which are used by the virus to penetrate cells and facilitate cell-to-cell spread, are known to trigger an efficient anti-viral immune response. The study was performed at APHA Weybridge, in tight collaboration with the Porcine Reproductive and Respiratory Syndrome Immunology Group at the Pirbright Institute. The results showed that the two vaccine candidates generated strong antibody and cellular immune responses in pigs. Since it is thought that both types of immune response are important for protection against Nipah virus, these results provide a solid basis for further investigation in vaccine development for use in pigs and potentially other animal species. Interestingly, BoHV-4 could also be used to make vaccines for other pig diseases where T cell responses are thought to play an important role in protection, such as African swine fever virus. The next step will be to assess whether they will protect pigs when they are infected with the Nipah virus after receiving the vaccine (challenge experiment). As emerging viruses continue to create new outbreaks around the world, preventing and controlling viral diseases that can spread from animals to people (known as zoonoses) has become a global priority. By preventing spread of the virus through pig populations, these novel types of vaccines would also lower the chances of humans contracting zoonotic diseases.

Publication: Pedrera, M.; Macchi, F.; McLean, R.K.; Franceschi, V.; Thakur, N.; Russo, L.; Medfai, L.; Todd, S.; Tchilian, E.Z.; Audonnet, J.-C.; Chappell, K.; Isaacs, A.; Watterson, D.; Young, P.R.; Marsh, G.A.; Bailey, D.; Graham, S.P.; Donofrio, G. Bovine Herpesvirus-4-Vectored Delivery of Nipah Virus Glycoproteins Enhances T Cell Immunogenicity in Pigs. Vaccines ***2020****, 8, 115.* DOI

Prof Gaetano Donofrio on his experience as TNA user:



"VetBioNet-TNA initiative gave me the great opportunity to test BoHV-4-based vector in sheep and pigs for Peste des petits ruminants and Nipah virus disease respectively, the latter one of a zoonotic nature._Further, I had the opportunity to know and interact with Dr. Simon Graham (Pirbright Institute, UK) and Dr. Veronica Martin (Centro de Investigación en Sanidad Animal, Spain). This interaction was humanly and scientifically productive. (…)

Probably the most noteworthy aspect of VetBioNet initiative was the ease with which to apply and in my opinion "science starts where bureaucracy ends".

For more information visit our website:

https://www.vetbionet.eu/

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