SHORT-TERM MISSION SUMMARY REPORTS 2016 – 2017

STM_3

Caroline Le Marechal of Anses Laboratory of Ploufragan-Plouzane, France, visited Dr Luca Bano of the Istituto Zooprofilattico Sperimentale delle Venezie, Italy, from 11 to 15 July 2016.

OBJECTIVE: The objective of this mission was to transfer specific technical skills between institutes regarding two different methods:

- Isolation of group III *C. botulinum* strains
- Detection of botulinum toxins with a focus on the Endopep-MS method

REPORT: During the mission, both naturally contaminated samples and pure cultures were used for training in these methods. Training covered sample analysis from receipt of sample to result analysis using various matrices.

Each step of the isolation process was presented (sample preparation, incubation, DNA extraction, PCR) and critical points of the method were highlighted: for example, a heat treatment at 71°C is required during the isolation process and using this specific temperature is critical to the success of isolation. It is also important to sample liquid at the bottom of the tube under meat particles, where most of the spores will be located. Given that media used for sample plating are not selective, recognition of the right colonies is also a main point in the process: colonies that should be picked are those with a pearly aspect, very small, positive for lipase and lecithinase when plating on EYA and weakly haemolytic when plating on BAB-2. Working on both naturally contaminated samples and pure culture was extremely instructive.

The novative Endopep-MS method which should become one of the reference methods for botulinum neurotoxin detection in the future was also presented. Different samples were used for the demonstration: supernatant from pure and mixed cultures, one intestinal content sample, and one pool of sera. Each step of the process was presented (sample preparation, BoNT capture using antibodies on beads, incubation with target peptides and detection of cleaved peptides using routine mass spectrometry).

I can now set up these methods in my laboratory.

STM_4

Apostolos Liakopoulos of Wageningen University (CVI), Lelystad, The Netherlands, visited Dr Teresa M Coque and Dr Val Fernández Lanza, of Ramón y Cajal University Hospital, and Dr Ricardo Ramos Ruiz at the Genomics Unit of the Madrid Science Park, Madrid, Spain, from 27 March to 9 April 2017.

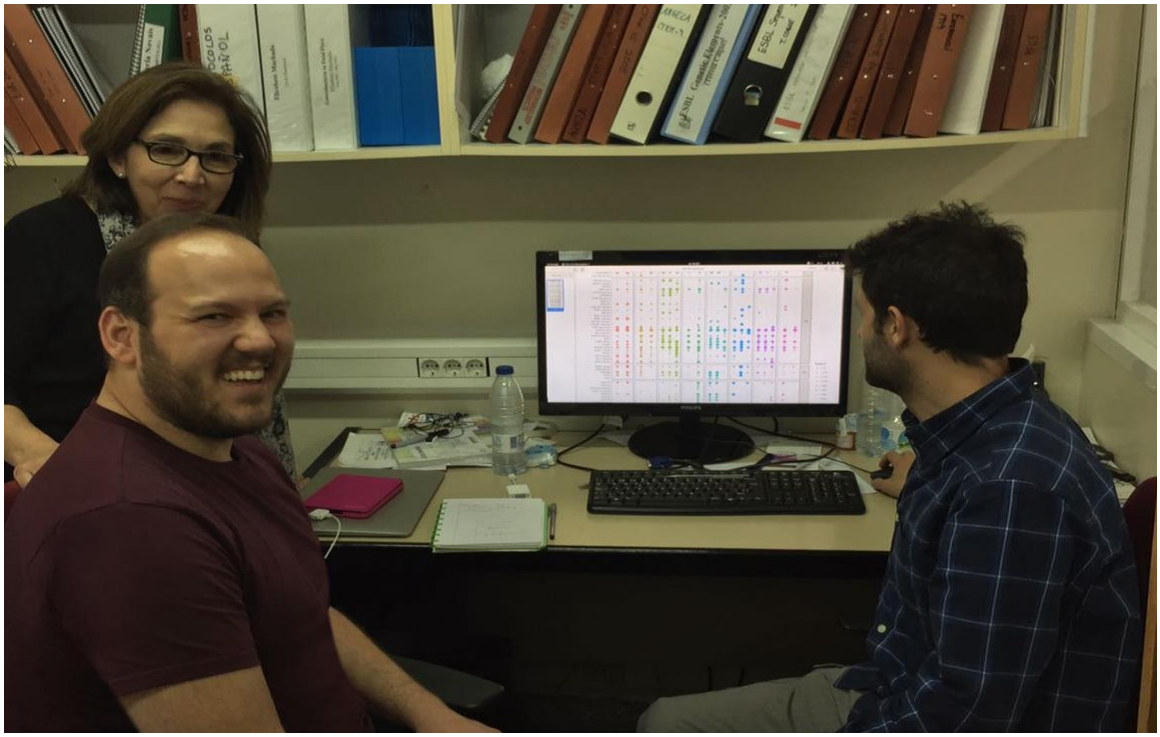
OBJECTIVE: Introductory traineeship in ResCap, a sequence capture platform that can be used for the enrichment of antibiotic resistant genes in minority populations of bacteria in complex biological systems.

REPORT: Antibiotic- and biocide- resistance genes have been found among bacteria of animal and human origin, as well as environmental ecosystems. These genes can be transmitted from animals to humans and vice versa through a variety of routes, highlighting the need to study their transmission within complex ecosystems, such as animals and humans from a 'One Health' perspective. Although, high-throughput DNA sequencing of metagenomes is currently used to describe the genetic diversity of bacteria in complex communities, antibiotic- and biocide- resistance genes are encoded only in minority bacterial populations in these communities and therefore their prevalence is below the threshold that metagenomic sequencing is able to detect. Here, we used ResCap, a sequence capture platform that can be used for the enrichment of antibiotic- and biocide-resistance genes in these minority populations of bacteria. The Rescap platform shows better recovery of the target genes than metagenomic shotgun sequencing and provides an estimate of their "abundance" within metagenomes. ResCap constitutes a valuable tool for comparative analysis of resistomes of samples of human and animal origin and subsequently facilitates the "One Health" approach of the global antibiotic- and biocide-resistant bacteria threat.

Team photos:

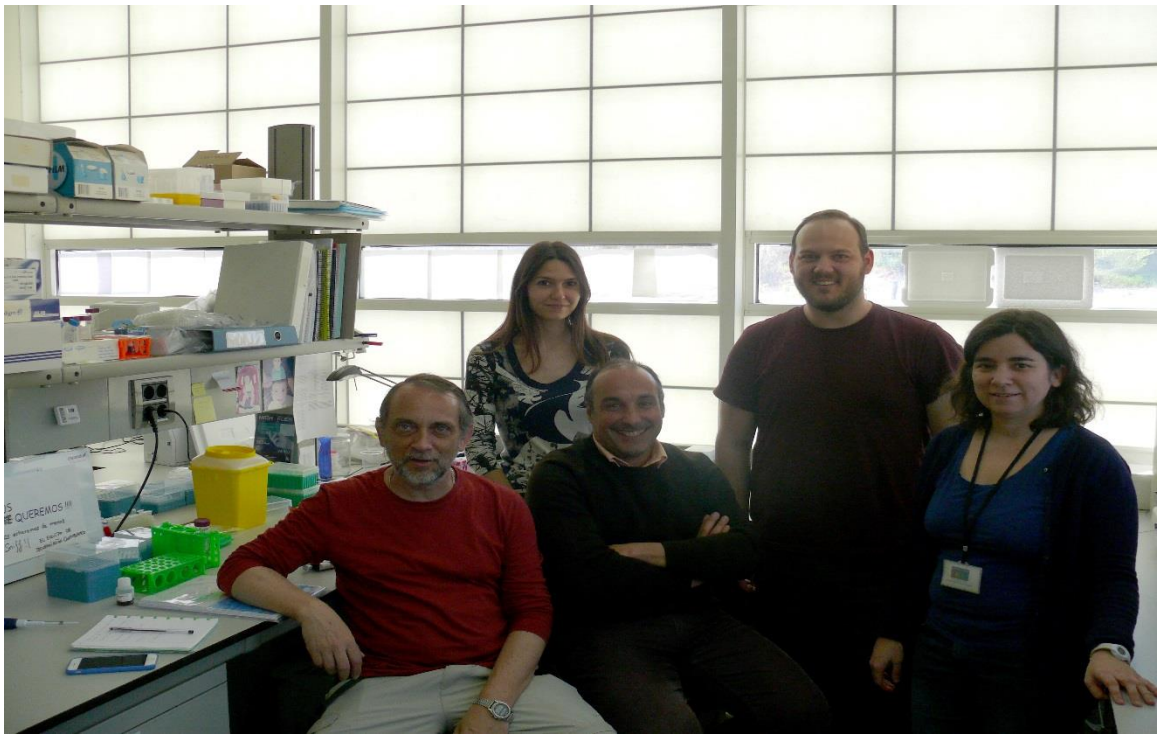
Department of Microbiology, Ramón y Cajal University Hospital (www.irycis.es)

From left to right (first row): Apostolos Liakopoulos, Val Fernández Lanza, Carmen de la Vega and , (second row): Javier de la Fuente-Hidalgo, Jerónimo Rodríguez-Beltrán, Maria Elena Barone, Teresa Maria Coque and Álvaro san Millán.



From left to right: Teresa Maria Coque, Apostolos Liakopoulos and Val Fernández Lanza.

Genomics Unit of the Madrid Science Park (www.pcm.es)



From left to right: Victor Fernandez, Silvia Vazquez, Ricardo Ramos Ruiz, Apostolos Liakopoulos, Susana Ovalle Andreu.

STM_5

Kenneth Klingenberg Barfod of Statens Serum Institut (SSI), Copenhagen, Denmark, visited Dr Michael J Cox, Imperial College, and Dr Frances Colles, University of Oxford, UK, from 3 to 9 September 2016.

OBJECTIVES: First, to attend a meeting for early career respiratory microbiome researchers organised by Michael J Cox in conjunction with The European Respiratory Society who came to London for the first time in September 2016 and as a result of this to find more international collaborations and plan a research stay. Second, to attend The 5th Exploring Human Host-Microbiome Interactions in Health and Disease meeting held at Cambridge UK from 7 to 9 September with the aim of learning new techniques and again to find potential collaboration partners.

REPORT: The financial support from MedVetNet allowed me to attend 3 different scientific and networking meetings in the greater London area. Attending these will be extremely important for my network and career development going forward. I was able to present my work and exchange ideas with leaders in the field. The recent years have seen an increasing interest in using probiotic bacteria as a means to control pathogens and zoonoses through the changes of the gut microbiota. The NGS approach to fight zoonoses and other infections is currently hampered due to a skill cleft between biologist and bioinformatics and I aim to be competent in both.

My current strategic career planning is steering towards an interdisciplinary position as a microbiologist with added independent skills in NGS methodology, immunology and the use of probiotics in animal production and care.

The support from MedVetNet has helped me gain 3 new collaborators from Spain, USA and Denmark along with a lot of other information. I have now been invited to make a short academic visit to the USA to learn specific protocols and I have an invite regarding future collaborations in transgenerational transfer of microbiota in animals from Spain. Finally, I gained insight and ideas that helped me finalize and refine my research direction resulting in a current grant application with the leading Danish animal welfare laboratory at the University of Copenhagen.

STM_6

Ana Victoria Ibarra Meneses of the Instituto de Salud Carlos III, National Center for Microbiology, Majadahonda 28220 Madrid, Spain, visited Dr Geraldo Gileno de Sá Oliveira at the Laboratory of Pathology and Bio-intervention (LPBI), FIOCRUZ, Bahia, Brazil, from 08 October 2016 to 08 December 2016.

OBJECTIVES: The main objective of this mission was the optimization of a whole-blood gamma interferon assay for the detection of *Leishmania infantum* in asymptomatic infected dogs and to evaluate the usefulness of the assay in the definition of susceptibility or resistance in canine visceral leishmaniasis.

REPORT: The whole blood assay was performed in dogs infected with *Leishmania infantum* (asymptomatic dogs) and healthy dogs. A cell proliferation assay (CPA) with soluble *Leishmania* antigen (SLA) was used as a reference test to detect asymptomatic dogs and healthy dogs from the kennel. Five dogs with CPA-positive (asymptomatic dogs) and three negative controls (CPA-negative) were tested.

SLA and recombinant antigens (NT-CT-rLci2 and rLci2-NT-CT-5R) stimulated plasma from asymptomatic dogs after 24, 48 and 72 h of incubation generated a higher production of cytokines in a short incubation time (24 h), thus being a quick test. When analyzing the different concentrations of antigens, we found that the best condition after the stimulus was 25 µg/mL SLA and 10 µg/ml for recombinant antigens. The highest concentrations were found in a fast time and with a low antigen concentration. Finally, when analyzing the undiluted and diluted plasma samples after SLA stimulation of whole blood we found that the highest concentrations of cytokines after 24-h incubation with 25 µg/mL of SLA and 10 µg/ml recombinant antigen were obtained using undiluted blood.

In conclusion, whole blood stimulation assay can be used as a tool to evaluate the cellular immunity of asymptomatic *L.infantum* infected dogs. In addition, the whole blood stimulation assay has been shown to be a rapid (24-h incubation), simple (whole blood), robust (low variability) and sensitive (high cytokine concentrations) tool, for use in the laboratory and in the field.

Team of Laboratory Pathology and Bio-intervention (LPBI). Fundação Oswaldo Cruz,- FIOCRUZ- Centro de Pesquisas Gonçalo Moniz, Salvador, Bahia, Brazil. Fernanda Gomes, Paula Dantas, Maria Carolina de Souza, Marcus David, Dr Geraldo Gileno, Ana Victoria Ibarra, Maria Carolina Gonçalves and Matheus Moreno.

STM_7

Laura Botana Veguillas of the Instituto de Salud Carlos III (ISCIII), Centro Nacional de Microbiología, Madrid, Spain, visited Anabela Cordeiro-da-Silva at the Institute for Molecular and Cell Biology, 4150 Porto, Portugal, from the 4th October to the 28th November 2016.

OBJECTIVES: The aim of our work was to determine the role of multifunctional T cells in *Leishmania infantum* infection in human samples, but some difficulties in optimising the protocol for human samples changed the main objective of this work. With the support of a Med-Vet-Net short-term mission grant, I learnt how to characterize multifunctional Th1 and Th2 cells in an experienced laboratory and characterize multifunctional Th1 and Th2 cells in infected mice. I will apply this method to study, in my thesis, for the first time a cohort of immunosuppressed patients with the technical skills learnt (intracellular staining, FACS,..) and the on-line support of Dr Cordeiro's lab which is delighted to collaborate with my work.

REPORT: As explained before, at first our aim was to determine the role of multifunctional T-cells in human visceral leishmaniasis, but by a series of unforeseen events we were not allowed to do. For this, we changed the main objetive and we determined the role of multifunctional T-cells in three different mice strains Balb/c (susceptible), C57BL/6 (susceptible) and SV129 (resistent) infected with *Leishmania infantum*.

In this work, we evaluated the intracellular production of IFN-γ, IL-2, IL-10 and TNF-α in spleen lymphocytes after two or eight weeks of infection (acute and chronic phase).

Two weeks after infection, infected Balb/c mice had higher levels of cytokine production when relatives frequences were compared with the other two groups. Eight weeks after infection, infected SV129 mice had lower levels of cytokine production when relatives frequences were compared with the other two groups. C57BL/6 had lower cytokine levels than Balb/c, but higher than SV129 in acute and chronic infection.

In conclusion, we have been able to characterize the intracellular cytokine profile of three different mouse lines after *Leishmania infantum* infection. This study has entailed a first personal approach to the study of intracellular cytokines.

Because cytokine studies in human *Leishmania* infection have been performed on culture supernatants, in near future we will optimize the protocol of intracellular cytokines in human samples and apply it in peripheral blood mononuclear cells from immunocompetent and immunosuppressed patients infected by *Leishmania infantum*, with the aim of better understanding the immunity of these patients to the parasite.

STM_8

María Victoria Ortega García of VISAVET-UCM, Madrid, Spain, visited Dr Francisco Javier Salguero Bodes of the Department of Pathology and Infectious Diseases at the Veterinary Pathology Centre and the School of Veterinary Medicine, University of Surrey, Guildford, UK, from 21 November 2016 to 21 February 2017.

OBJECTIVES:

- To obtain laboratory experience and learn the theory behind new techniques based on direct fluorescent antibody assay and immunohistochemistry, that are very useful in the detection of *Leishmania* species and Leishmaniosis research.
- To learn about the new technique of laser capture microdissection not as yet used in VISAVET but that could be used for research collaboration in the future as well.
- To obtain a European mention in my doctorate.

REPORT: This project has provided the applicant with the necessary knowledge and practical skills (hematoxylin and eosin stain, epitopes unmasking, immunofluorescence and immunohistochemistry techniques) to perform the histological and immunofluorescence examination of different tissues from wild *Leporidae*, as well as the immunohistochemical evaluation of tissues from other animals such as horses, dogs and cats, in order to detect *Leishmania* sp. parasites in mammalian hosts. Furthermore, laser capture microdissection (LCM) has been carried out to obtain dissected lesions of different stages from selected tissue sections for an eventual nucleic acid extraction. This study has given the applicant the opportunity to acquire more experience in the most cutting-edge techniques in Veterinary Pathology Diagnostics.

STM_10

Rachel Taylor of the Animal and Plant Health Agency, Addlestone, Surrey, UK, visited Dr Gert Jan Boender, Dr Aline de Koeijer and Dr Peter Hobbelen at Wageningen Bioveterinary Research, The Netherlands, from 27 to 31 March 2017.

OBJECTIVE: Applying spatial modelling methods to risk assessment.

REPORT: Wageningen Bioveterinary Research has considerable expertise in spatial modelling of disease outbreaks and associated risk of future outbreaks. Rachel used this short-term mission to quickly and intensively learn the key methods, as well as its potential problems and how to overcome them. In particular this method fits a transmission kernel of disease spread from location to location based on previous outbreak data. It can then be used to assess risk of locations spreading disease for future outbreaks.

Through discussion of the method and outlining of the current code in Mathematica, Rachel was introduced to the fitting procedure for creating the kernel. To have the

most success at learning how to do this herself, Rachel programmed the algorithm in the open source language R. This allowed for Rachel to understand each step in the process and how it might be applied to different outbreak and data scenarios. Thus, the method can be used by Rachel in the future for different diseases (including zoonotic disease), both to assess disease spread of current outbreaks and risk for future outbreaks. Furthermore, Rachel used R instead of Mathematica (used by the host institute), allowing speed comparisons to be made, and therefore helping the host institute assess their own code.

STM_11

Hengameh Chloé Lauridsen of Statens Serum Institut (SSI), Copenhagen, Denmark, attended Digestive Disease Week 2017, Chicago, IL, USA, from 6 to 9 May 2017.

The workshop provided the opportunity to meet Dr Hongbing Yu and Dr Hyungjun Yang, collaborators from the Bruce A. Vallance laboratory, Vancouver BC, Canada.

OBJECTIVES: Aquiring knowledge from our collaborator in order to establish an *in vivo*- imaging system in our lab; and gaining knowledge of how to clone and express luminescence (*lux*) on the bacterial chromosome, in order to be able to establish this method in our lab at Statens Serum Institut, Denmark.

Presenting our research results at the Digestive Disease Week 2017 conference, based on an Inflammatory Bowel Disease (IBD) mouse-model-colonization with IBD-associated *E. coli*.

REPORT: We establish a mouse model of chronic intestinal infection by the Ulcerative colitis (UC) derived *E. coli* pathobiont p19A. This study was performed in collaboration with Bruce A. Vallance at the Department of Pediatrics, BC Children's Hospital, University of British Columbia, Vancouver, Canada. The experimental mouse work was carried out at the laboratory of Dr Bruce A. Vallance.

Mice lacking the single immunoglobulin and toll-interleukin one receptor (TIR) domain ((SIGIRR) (Sigirr $^{-/-}$)) were chosen. Mice were pre-treated with vancomycin for 6 h before infection with bacteria. The next day, 3 % DSS was added to their drinking water for 4 days to induce colitis. **UC associated *E. coli* p19A WT expressing luminescence (*lux*) on chromosome** and non-pathogenic lab *E. coli* DH10B were cultured in LB broth at 37°C, 200 rpm overnight, and 100 µl of subculture was used for oral gavage into mice. Mice were monitored every day and euthanized on the fifth day after infection. Colonization with p19A WT was visualized by an **in-vivo imaging system** (before and after washing the luminal contents). Our findings provide evidence that UC-associated phylogroup B2 *E. coli* strain p19A WT can readily and persistently colonize the intestines of susceptible hosts, and significantly worsen the course of colitis.

At this workshop, I gained the knowledge to enable me to introduce this *in-vivo* imaging system at our lab and to clone and express luminescence (*lux*) on the bacterial chromosome. This system will be used in our future studies at our lab, Statens Serum Institut, Denmark.