Project: **Segment-2 sequencing and analysis of Bluetongue virus isolates (Ref: PIR7)**

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Research Group: **Non-Vesicular Reference Laboratory**

Project Summary:

The primary purpose of the Non-Vesicular Reference Laboratory (NVRL) is to provide a diagnostic service and characterise outbreaks of livestock diseases caused by Bluetongue virus (BTV), African horse sickness virus, African swine fever virus, Peste des Petits Ruminants Virus, Rinderpest virus and Capripox viruses. As European Union Reference Laboratory (EURL) for BTV, the group maintains a unique collection of virus isolates. The NVRL also performs applied research such as the development and validation of new diagnostic assays and vaccines, as well as investigating the molecular epidemiology of arboviruses. The placement student will be expected to perform partial sequencing of the BTV genome under the supervision of a senior staff member. The student will be trained on selected molecular methods, including nucleic acid extraction, conventional and real-time PCR and he/she will be expected to work independently in the molecular suite after obtaining the relevant training. In addition, the student will support the NVRL in routine duties such as housekeeping, consumable management, sample reception and other duties as required. This placement will provide the successful student with a unique opportunity to work in an ISO/IEC 17025-accredited Reference Laboratory and to gain practical knowledge of sequencing techniques.

Details:

BTV is the causative virus of bluetongue (BT) disease, an infectious disease of ruminants, transmitted mainly by biting midges (*Culicoides* spp). BT is a World Organization for Animal Health (OIE) reportable disease and it is of considerable socioeconomic concern and of high importance in the international trade of animals. Before 1998, BT was considered an exotic disease of the UK, but since has caused a number of outbreaks across Europe. Currently, BTV serotype 8 (BTV-8) has been detected in France as part of active surveillance and the risk of BTV incursion to the south coast of England seems likely. BTV serotypes such as BTV-1, BTV-2, BTV-4, BTV-8, and BTV-16 have been continuously present in many countries of the Mediterranean with BTV-4 spreading north to Croatia, Slovenia, Bulgaria, Austria, Romania, Hungary, and Slovakia.

BTV belongs to the family *Reoviridae,* genus *Orbivirus* and is characterised by a high genetic diversity and the existence of multiple serotypes (over 27). The BTV genome consists of 10 double-stranded RNA segments that encodes for structural (NS1, NS2, NS3 and NS3A) and non-structural (VP1- VP7) proteins. The structural protein VP2, encoded by the most variable region of the virus genome (segment 2), is located at the surface of the virion and is the major target of the host immune response. A high genetic viability observed in BTV is attributed to evolutionary forces such as point mutation and reassortment of individual gene segments.

As EURL, the NVRL receives specimens from many countries affected by BT disease for differential diagnosis and characterisation. Real-time RT-PCR testing, serotyping by real-time RT-PCR, virus isolation and sequencing of segment-2 of the BTV genome is performed in the NVRL on a regular basis. BTV isolates are fully characterised by sequencing, mycoplasma testing and virus titration prior to addition to the reference collection. BTV segment 2 sequencing provides valuable epidemiological information which is required for a better understanding of BTV origin and spread and will contribute to outbreak investigations and control strategies.

During this project, the placement student will perform BTV propagation using KC and BHK cells, extract viral dsRNA using TRIZOL reagents, and produce cDNA using the anchor-primer method. The placement student will be trained in sample preparation for Sanger sequencing, and in data analysis such as multiple sequence alignment, selection of an appropriate evolution model, building a phylogenetic tree using different methods with MEGA software, R and Bioconductor.

References for Suggested Reading:

- Maan, S. et al (2007) Rapid cDNA synthesis and sequencing techniques for the genetic study of bluetongue and other dsRNA viruses. Journal of Virology methods; 143(2):132-9
- Maan, S et al (2012) Identification and Differentiation of the Twenty Six Bluetongue Virus Serotypes by RT–PCR Amplification of the Serotype-Specific Genome Segment 2. PLOS ONE 7(2)