REF: 05/PF/PB

Project Title: Characterising the extracellular virions of lumpy skin disease virus (LSDV)

Supervisors: Dr Petra Fay and Dr Pip Beard **Research group:** Large DNA Viruses (PoxWorld)

Project Summary:

Poxviruses undertake a complicated replication cycle which incorporates the production of four types of virions – intracellular mature virions (IMVs), intracellular enveloped virions (IEVs), cell associated enveloped virions (CEVs) and extracellular enveloped virions (EEVs). EEVs consist of IMVs wrapped in an extra membrane. For the orthopoxvirus genus (OPXV) it has been shown that IMVs are by far the predominant virion type produced with EEVs forming around 1% of the total. However EEVs are crucial to the pathogenesis of OPXVs as they allow the virus to spread to distant sites within the host. Very little is known about EEV production by CPPVs, however recent confocal and electron microscopy studies by the PoxWorld team have uncovered preliminary evidence that the morphogenesis of CPPVs is similar to OPXVs, with IMVs, IEVs, CEVs and EEVs identified in LSDV-infected cells, along with other key features of poxvirus replication (viral factories, wrapping stations and actin tails).

Details:

Work Package (WP) 11 of the DEFEND programme (https://defend2020.eu/) concentrates on LSDV, a member of the capripoxvirus (CPPV) genus. LSDV causes a severe systemic disease in cattle, and is endemic throughout Africa. Better vaccines are required to prevent further spread of the disease into Europe, and this is the focus on the WP. It is likely that these novel LSD vaccines will need to specifically target EEVs in order to be efficacious, similar to the situation for OPXVs. This placement student project will characterise the EEVs produced by LSDV in order to guide the development of improved LSDV vaccines. The main objectives of the project are:

1. Compare orthopoxvirus and capripoxvirus EEV proteins using **bioinformatics tools**. (20%)
   EEVs produced by OPXVs are known to contain between 4 and 6 additional viral membrane proteins compared to IMVs. A further 3 viral proteins are required for OPXV IEV and CEV production. A bioinformatics analysis (using MEGA and Clustal software packages) of the CPPV orthologues of the 9 genes encoding these proteins will be undertaken in order to predict whether CPPVs are likely to produce EEVs in a fashion analogous to OPXVs.
2. Determine and quantify the production of LSDV EEV. (50%)
   LSDV will be used to infect MDBK cells, and EEVs quantified by virus detection methods including confocal microscopy, western blotting, plaque morphology analysis and similar techniques.
3. Characterise LSDV EEV production. (30%)
   The production of EEVs requires activation of a pathway involving Src and Abl family kinases. The role of these pathways in the production of LSDV EEVs in primary bovine cell lines will be studied.

The student will be directly supervised by Dr Petra Fay and Dr Pip Beard and will attend the relevant DEFEND project team meetings. They will also be supported by the rest of the PoxWorld team. The student will be trained in all of the techniques and methods required to complete the project. Some of this will be provided by the Institute, but most will be hands-on in the lab. The student will be trained to work in high containment, will gain experience of cell culture as well as the techniques outlined above.

References for Suggested Reading:

- Beard, P. M. (2016). "Lumpy skin disease: a direct threat to Europe." Veterinary Record **178**(22): 557-558.
- Law, M. and G. L. Smith (2001). "Antibody neutralization of the extracellular enveloped form of vaccinia virus." Virology **280**(1): 132-142.
- Newsome, T. P., Weisswange, I., Frischknecht, F. and Way, M. (2006), "Abl collaborates with Src family kinases to stimulate actin-based motility of vaccinia virus". Cellular Microbiology **8**: 233-241. doi:10.1111/j.1462-5822.2005.00613.x