REF: 03/MG/KD

Project Title: Understanding the occurrence of genome exchange between different strains of Bluetongue virus following the infection of the same target cells

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Project Summary:

Bluetongue virus (BTV) is transmitted between its ruminant hosts (sheep, cattle, goats, deer) by *Culicoides* biting midges. In ruminants BTV causes a severe haemorrhagic and economically important disease (called bluetongue). The virus genome is divided into 10 segments of double stranded (ds)RNA. When two strains of BTV are infecting the same cell, genome-segments can be exchanged between both viruses and this process is called reassortment. This can lead to the emergence of new viral strains (reassortant strains), which may have gained new traits (e.g. causing more severe disease or being transmitted more efficiently etc.)

Interestingly, historic research has suggested that if infections occur not at the same time but one after the other (asynchronously), the initial infection by the first virus might block/exclude the infection by a second BTV strain (superinfection) in a time-dependent manner. Superinfection exclusion during asynchronous infections would thereby restrict genome reassortment of BTV to a precious yet currently undefined time window. This project will mainly investigate superinfection exclusion, and will therefore help to get a better understanding of when and how BTV genome reassortment occurs, an event still poorly understood.

Details:

In the field, we know that genome reassortment between different BTV strains occurs frequently. However, it seems far more likely that infection of the same animal or insect vector with two different BTV strains would occur asynchronously. Confirming and characterising superinfection exclusion in BTV is therefore vital to understand how BTV genome reassortment occur in the field.

Until recently, one of the main limitations has been the inability to demonstrate that two BTV strains have entered the exact same cell and not merely different individual cells in the same cell culture. However, recent technological advances allow for the design of fluorescent tagged molecular probes which can be designed to detect specific genome areas sufficiently different between two BTV strains. The successful candidate will initially learn how to design virus genome specific molecular probes and verify that the molecular probes can identify and quantify the occurrence of both virus strains infecting the same cell. The student will then carry out asynchronous infection time courses with both BTV strains in several cell lines and investigate the occurrence of co-infection and superinfection exclusion by detection of respective fluorescent probes using flow cytometry and confocal microscopy. Additionally the production of newly generated virus from each respective strain will be quantified in culture supernatant by gene specific qPCR. Alternatively, BTV serotype specific antibodies are also available for the detection of dual infection in the same individual cell.

The student will join the Orbivirus Research group, whose main interest is the study of host-virus-insect vector interactions of *Culicoides*-borne viruses. All the necessary training, including how to work in high containment, will be provided within the group and the Institute, and the student will be directly supervised by a postdoctoral scientist with strong expertise in molecular biology. The student will join a highly collaborative and vibrant research environment and have excellent opportunities to develop technical competence and valuable experiences in key scientific areas of molecular virology and cell biology.

References for Suggested Reading:

- EL HUSSEIN, A., RAMIG, R. F., HOLBROOK, F. R., & BEATY, B. J. 1989. Asynchronous Mixed Infection of *Culicoides variipennis* with Bluetongue Virus Serotypes 10 and 17. Journal of General Virology, 70, 3355-3362.
- NOMIKOU, K., HUGHES, J., WASH, R., KELLAM, P., BREARD, E., ZIENTARA, S., PALMARINI, M., BIEK, R. & MERTENS, P. 2015. Widespread Reassortment Shapes the Evolution and Epidemiology of Bluetongue Virus following European Invasion. PLoS Pathog, 11, e1005056.
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- RAMIG, R. F., GARRISON, C., CHEN, D. & BELL-ROBINSON, D. 1989. Analysis of Reassortment and Superinfection during Mixed Infection of Vero Cells with Bluetongue Virus Serotypes 10 and 17. Journal of General Virology, 70, 2595-2603.