Report on STM to CReSA/IRTA, Barcelona, 2-29 August 2017
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Thanks to the support of EPIZONE short term mission, I visited CReSA/IRTA, Barcelona, Spain, during 2-29 August 2017. I have learnt techniques in studying African swine fever virus (ASFV) that are routinely applied at Dr. Fernando Rodríguez's lab. I have achieved majority of my objectives, while some parts couldn't due to certain circumstances, for examples an ongoing large experimental infection with reduced number staff (in August) processing large number of samples collected from animals. I have practiced all the techniques during the visit and here is a brief description of the achievements:

1. Virus isolation on macrophages. It was not possible to harvest macrophages from pig lungs this time, so macrophages from previous experiments were used to grow ASFV BA71 strain and a vaccine candidate BA71ΔCD2. The latter was attenuated from BA71 and with a cross-protection capability against not only genotype I viruses but also genotype II (Monteagudo et al., 2017).

2. Virus titration is fundamental for any experiments. Depending on virus strain, virus titer can be determined by: (A) *Plaque assay*, which is measure infectious lytic viral particles on agar-covered cells where viral infection forms plaques. Both BA71 and BA71DCD2 formed plaques on macrophages. (B) *Hemadsorption assay* (HAD), which relies on the viral CD2 protein CD2v to attach to red blood cells. HAD was used to titrate BA71 strain but not the vaccine candidate BA71DCD2. (C) Immuno-peroxidase assay (IPMA), which is able to titrate virus that does not cause measurable cytopathic or lytic effects on cells, and is expressed by $TCID_{50}$. (D) real-time PCR assay is useful when none of the above assays works for a strain.

3. Isolation of peripheral blood mononuclear cells (PBMCs) using Histopaque-1077 (Sigma, Cat. No. 10771). PBMCs contain immune cells and are of great importance in studying host response to virus infection or vaccination. Histopathology and microscopic evaluation of tissue samples were not possible due to high work load at that moment.

4. Fluorescence-activated cell sorting (FACS) is a powerful tool to analyze of different cell populations in PBMCs. We found that the proportions of CD8+ and CD4+CD8+ T cells in PBMCs from vaccinated animals increased upon stimulation with BA71 and BA71DCD2. We also did FACS on macrophages infected with BA71 and BA71DCD2.

5. ELISPOT to measure any cytokine response of the PBMCs upon stimulation with either viruses or peptides for which fresh PBMCs have encountered previously. Here we measured IFNγ response upon stimulation with different ASFV strains and peptides.

Besides these techniques very useful for ASFV studies, I also got to know how works are organized and good cooperation among teams. All the outcomes will be very useful for me to do ASFV research and to manage project/laboratory in future.

Reference:
Monteagudo PL, Lacasta A, López E, Bosch L, Collado J, Pina-Pedrero S, Correa-Fiz F, Accensi F, Navas MJ, Vidal E, Bustos MJ, Rodríguez JM, Gallei A, Nikolin V, Salas ML, Rodríguez F. BA71ΔCD2: A new recombinant live attenuated African swine fever virus with cross-protective capabilities. J Virol. 2017 Aug 16. pii: JVI.01058-17. doi: 10.1128/JVI.01058-17. [Epub ahead of print]