**Dr. Arta LUGAJ**
Agricultural University of Tirana,
Kamez Street, Tirana
T: 00355 692735648
E: lugajarta@gmail.com

## Scientific Report

This Scientific Report is focused on the outputs that I gained during the training period in Madrid Spain, from 17.03.2018 to 15.04.2018, in three different institutes CISA-INIA-INGENASA-UCM. The main topic of this scientific research work has been more focused on learning general and basic information about African Swine Fever disease (ASF) and the second aim was to be informed about the current situation of ASF in Eastern Europe. As in Albania ASF is not present in domestic pigs and wild boar, this training was very useful to have an overview about the ASF disease and to be prepared in the future if the disease will occurred in Albania.
During my stay in Madrid I assisted in three different Institutions as mention below:

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| **1. Name of Institution:** | **INGENASA** |
| **2. Address:** | **Av. de la Institución Libre de Enseñanza, Hermanos Garcia Noblejas, 41 Madrid 28037 Spain** |
| **3. Training Period time:** | **19.03.2018-23.03.2018** |
| **4. Lead Scientist** | **Dr. Paloma Ruedes** |
| **5. Molecular Techniques performed:** | **DAS ELISA, Blocking ELISA, LUMINEX, Lateral Flow Assay, Dublex Lateral Flow** |

At INGENASA I performed: ***Sandwich ELISA*** for detection of viral protein VP 72 of ASFV in serum sample, collected from infected pigs. We used the kit and the protocol from DAS ELISA INGENASA. We created also the ELISA curve, after testing a serial dilution from infected sera with ASFV.
***Blocking ELISA*** for detection of specific antibodies anti vp73 of ASFV (structural protein with high antigenic power) from sera in infected pigs that were collected in different days post-infection. ELISA plates were covered with vp73. After the sera addition, a monoclonal antibody anti-vp73 was added, marked with peroxidase to cover the free plates, where no antibodies have done the antigen-antibody fixation. The interpretation of the results is opposite as the previous ELISA. More color means less level of antibodies in sera samples, and vice versa.
***LUMINEX assay*** is used for detection of different and several analytes only in one well, in comparison to ELISA assays that can detect only one analyte in one well. We performed four different analytes in which three of them were from African Swine Fever VP72, VP 50, VP 12 and one Viral Protein of Classical Swine Fever Virus as it is described below:
The sample is added to a mixture of color-coded beads, pre-coated with analyte-specific capture antibodies. The antibodies bind to the analytes of interest. Biotinylated detection antibodies specific to the analytes of interest are added and form an antibody-antigen sandwich. Phycoerythrin (PE)-conjugated streptavidin is added. It binds to the biotinylated detection antibodies. Polystyrene beads are read on a dual-laser flow-based detection instrument, such as MagPig Luminex. One laser classifies the bead and determines the analyte that is being detected. The second laser determines the magnitude of the PE-derived signal, which is in direct proportion to the amount of analyte bound. Magnetic beads can be read using the Luminex MAGPIX® Analyzer. A magnet in the MAGPIX analyzer captures and holds the magnetic beads in a monolayer, while two spectrally distinct light-emitting diodes (LEDs) illuminate the beads. One LED identifies the analyte that is being detected and, the second LED determines the magnitude of the PE-derived signal. Each well is imaged with a CCD camera.
***Lateral Flow Assay*** is a rapid test similar to a pregnant test in order to be used in field, to have a quick answer were the sera are tested for detection of Antibodies or Antigens. At INGENASA I performed two LFA assays for detection of Antibodies: 1. DR (double recognition) and 2. Indirect (double migration). and for detection of Antigens I used two other methods such as: 1. DAS (double antibody sandwich) and 2. Competition (detection of small molecules).

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| **Name of Institution:** | **CISA-INIA** |
| **Address:** | **Valdeolmos, 28130 Madrid** |
| **Training Period time:** | **26.03.2018-13.04.2018** |
| **Lead Scientist:** | **Dr. Ana De La Torre, Raquel Nieto** |
| **Department:** | **Epidemiology Department and EURLASF (European Reference Lab of ASF BSL3)** |

In this period I learned about the Epidemiology of ASF, about the past distribution of ASF occurred in 1957, from West Part of Africa to European Continent in Portugal and the present distribution of ASF from East part of Africa to Georgia (2007) – Armenia – Azerbaijan - Russia (2008) – Belarus (2012) – Ukraine (2013) [*Non-European Union Countries*] – Lithuania (2014) - Poland (2014) - Latvia (2014) – Estonia (2014) – Rumania (2017) –Czech Republic (2017) [*European Union Countries*].
I learned about the theoretical part of ASF and ASFV based on molecular biology of the virus ASFV, general information of the ASF non zoonotic disease, the host of ASF, Eradication program used in the past outbreak of ASF in Spain, Serological diagnosis, Risk Assessment, the distribution of ASF associated with the distribution of Wild Boar etc.
I assisted also a week at Bio Safety Level 3 Lab BSL3 EURLASF (European Reference Rab of ASF) at CISA-INIA and I performed different techniques for detection of Antibodies and Antigens testing different kind of samples such as sera, blood, liver spleen, kidney and lung as it is described below:

1. Procedure for African Swine Fever Virus (ASFV) isolation on porcine leucocytes and hemadsorption test.
2. Standard operating procedure for samples processing for African Swine Fever (ASF) diagnosis.
3. Standard operating procedure for the detection of antibodies against African Swine Fever by Blocking ELISA.
4. Standard operating procedure for the detection of antibodies against African Swine Fever by immunoblotting.
5. Standard operating procedure for the detection of antibodies against African Swine Fever by indirect Immunoperoxidase technique IPT.
6. Standard operating procedure for the extraction of African Swine Fever Virus (ASFV) DNA.
7. Standard operation procedure for the detection of African Swine Fever Virus (ASFV) by Real-Time Polymerase Chain Reaction (PCR) using universal probe library (UPL).
8. Standard operating procedure for genotyping of African Swine Fever Virus (ASFV) isolates.

I have learned and performed these different molecular techniques, for detection of ASF Antibodies and Antigens testing different serum samples and in the end of these assays we described the diagnoses of animals based on the results of these serological assays.

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| **Name of Institution:** | **UCM** (Complutense University of Madrid) |
| **Address:** | **Av. Séneca, 2, 28040 Madrid, Spain** |
| **Training Period time:** | **06.04.2018** |
| **Lead Scientist:** | **Prof.Dr. Jose Manuel Sanchez Vizcaino** |

On 06.04.2018 I had a presentation at UCM (Complutense University of Madrid) with Professor Jose Manuel Sanchez Vizcaino, a scientific expert of ASF. My presentation was about the scientific work that I performed during my training period in Madrid-Spain at INGENASA and CISA-INIA.
Attached you may find my presentation.

Sincerely,

**Dr. Arta LUGAJ**

***Dra. Ana de la Torre Reoyo***

***Epidemiología y Sanidad Ambiental***
***Centro de Investigación en Sanidad Animal (CISA-INIA)***

***Agricultural University of Tirana, Albania.***
***Faculty of Veterinary Medicine***
***Department of Veterinary Public Health***
***E-mail: lugajarta@gmail.com***