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EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

SHORT TERM SCIENTIFIC MISSION (STSM) SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: CA15116 – Understanding and Combating African Swine Fever In Europe (ASF-STOP)
STSM title: African Swine Fever virus transmission studies in *Ornithodoros verrucosus*
STSM start and end date: 06/11/2017 to 21/11/2017
Grantee name: Serhii Filatov

PURPOSE OF THE STSM:

The STSM was carried out at ANSES-Ploufragan Laboratory of Pig Virology and Immunology (VIP) under the supervison of Dr. Marie-Frédérique Le Potier. The purpose of this activity was to (i) conduct ASFV transmission experiments with *O. verrucosus* soft ticks; (ii) to receive practical training in methods and techniques used for the transmission experiments, assessment of infectious status and quantification of the infection in ticks and pigs.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

The STSM was carried out according to the proposed work-plan. During the two weeks of my stay in Ploufragan I participated in tick-transmission trials for African swine fever virus to naïve pigs. The transmission experiments were conducted with three different tick species (*O. moubata, O. erraticus and O. verrucosus*) and four ASFV isolates (LIV13/33, OURT-88/1, Georgia 2007/1, Ukraine/Zapo-12). During the first stage of the experiments in September-2017, ticks were fed on viraemic pigs (acquisition feeding). After two month, in November-2017 groups of infected ticks were fed on naïve SPF pigs in order to assess if they will be able to transmit the virus (transmission feeding). There were five groups of tick-virus couples used for simple transmission (TS: O. moubata-LIV, O. moubata-Georgia, O. erraticus-OURT, O. erraticus-Georgia, O. verrucosus-Zapo; single challenge with 30 ticks per animal) and two groups used for the repeated transmission attempts (TC: O. erraticus-Georgia, O. moubata-Georgia; 3 challenges with the interval of 3 days, 15

ticks per animal). Immidiately after the feeding, ticks were sorted according to the engorgement status (gorged, non-gorged), sex and stage. The engorged adult ticks in each group were placed together in tubes to allow for mating and oviposition. After the oviposition (~14 days), eggs in each tick-virus group were taken out and split into two pools to detect the virus and quantify filial infection rates respectively. Presence of ASFV in tick excretions (coxal fluid) deposited on mosquito netting used in feeding units during the challenge experiments was quantified by qPCR. Also, later in the course of experiments, 10 individual engorged ticks from three TS group were washed 3 times in PBS, homogenized and mixed with the RPMI medium. Portions of the resulting homogenates (200 µl/tick) were assayed for ASFV by qPCR using uiversal probe library and the rest (800 µl) was used for the virus isolation assays and inoculation of the previously challenged but uninfected animals.

After the tick challenges pigs were tracked for hyperthermia and other clinical signs of ASF daily. Blood samples to detect presence of the virus (by qPCR) were collected from the animals twice a week. Diseased pigs were euthanized at the peak viremia level, blood and tissue samples upon necropsy (tonsils, spleen, mesenteric lymph node) were taken to quantify virus loads.

During my visit to ANSES I participated in all of the above mentioned activities.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

High feeding success (up to 100%) was observed in all groups of infected ticks. By the day 13 of the experiments, females of O. moubata and O. erraticus laid eggs, which have been pooled for the subsequent analysis. There were no eggs in tubes with O. verrucosus by this date however, this species usually starts oviposition 2-3 weeks after a blood-meal. Moreover, in this group we had less adult ticks than in others. Hence, it is expected to obtain at least some eggs later in the course of the study in order to assess the possibility of transovarial transmission of ASFV in O. verrucosus.

Simple transmission (TS) experiments were successful in couples Moubata-LIV and Moubata-Georgia, with animals developing hyperthermia on Day 2 and Day 3 after the challenge, respectively. All animals in these groups (including contact ones) were euthanized by the Day 4 of the experiments. Animals in other TS groups remained apyretic, with no detectable virus in blood during the entire period of observations despite that ASFV genome was detected on mosquito nets from all but one (Moubata-Georgia) feeding units. Later, viral genome quantification assays with tick homogenates showed that both species which failed to transmit ASF to animals had quite high virus loads with the following mean Ct values: 26.85 (O. erraticus/Georgia), 24.85 (O. erraticus/OURT) and 29.16 (O. verrucosus/Zapo). Viability of the detected virus is currently being assessed by virus isolation on porcine macrophage cells. Depending on the results of this assay, it will be decided whether it is necessary to inoculate the animals in these groups. Contrary to the TS group, both O. erraticus and O. moubata failed to transmit Georgia isolate to

susceptible animals in the repeated challenge (TC) experiments.
During the STSM I gained extensive hands-on experience with methods and techniques used to carry out ASFV transmission experiments in high biocontainment settings (BSL-3). Also, I observed differences and similarities in physiology (e.g. different character of feeding lesions, amount of a bloodmeal, time of coxal fluid excretion) between 3 soft tick species. Moreover, I had the opportunity to observe clinical ASF and gross lesions in animals.

FUTURE COLLABORATIONS (if applicable)

It is planned to conduct further series of experiments on ASFV transmission with the 3 soft tick species used in the present study. The results of the STSM together with further work will be published in a peer-reviewed journal.

I herein approve the present report regarding the COST-STSM-CA15116-38749 at ANSES-Ploufragan Laboratory of Pig Virology and Immunology

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