

Title: Evaluation of African Swine Fever virus nucleic acid detection dynamics in swine in oral fluid samples collected after the detection of the presence of the ASF virus in pigs from commercial establishments – **NPB #21-067**

Investigator: Prof. Andrei D. Mihalca

Institution: University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

Date Submitted: 15 Oct 2021

Industry Summary: Acquiring ASF positive samples from an endemic country to test at FADDL will help to determine how these samples types and diagnostic tests can be utilized during an ASF response in the US. Samples and information will come from Romanian farms that mirror US production in housing configuration and genetics. There is an opportunity to get onto farms immediately post-infection to collect samples and information. Communication with both Romanian government officials as well as veterinarians on production sites demonstrates high Romanian motivation for collaboration. In addition, the probability of commercial sites becoming infected in Romania is high, so the expectation is that samples will be available to be shipped to FADDL for testing. The diagnostic results can then be placed into models to demonstrate how we can utilize these samples and test results in the US during an ASF outbreak to help develop and refine our surveillance protocols. Animal health officials, diagnostic laboratories, veterinarians, and producers are all working to prepare for an ASF outbreak and having surveillance protocols prior to an outbreak will help drive that effort.

Key Findings:

- Overall, the following samples have been collected, labelled, packed and stored at -80 C:
 - 3000 blood samples collected from three farms (1000 in each farm). They were packed in stands of 100 tubes (8.5 ml each)
 - 197 samples of saliva, packed in 50 ml falcon tubes. All tubes were stored in zip lock bags at -80 C
 - 8 samples of spleen, stored in zip lock bags at -80 C

Keywords: pigs, African swine fever, Romania, blood samples, saliva

Scientific Abstract: 3000 blood samples, 197 saliva samples and 8 spleen samples were collected, transported, stored (-80 C), packed, labeled and shipped to USDA between August and October 2021.

Introduction: In order to validate diagnostic tests and establish surveillance protocols for use in the United States prior to an African swine fever (ASF) outbreak, researchers at the Foreign Animal Disease Diagnostic Laboratory (FADDL) located at Plum Island need access to positive ASF samples.

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

For more information contact

National Pork Board • PO Box 9114 • Des Moines, IA 50306 USA • 800-456-7675 • Fax: 515-223-2646 • pork.org

cause ASF does not currently infect animals in the United States, one option would be to acquire positive ASF samples from other countries which do have ASF positive cases in their commercial swine herds. These samples could be collected from commercial farms in the infected country and shipped to FADDL at Plum Island for evaluation and testing. Collaborating with officials in Romania will help US producers by acquiring ASF positive samples collected post-outbreak. The purpose of this objective is to evaluate spread of the ASF virus through a commercial farm in Romania within a 48-hour time period. After a presumptive positive is identified at a commercial site, and before confirmation and depopulation, veterinarians in Romania will respond quickly to access farms and collect oral fluid and whole blood samples. Dr. Alexandru Supeanu, veterinarian with the Romanian Sanitary Veterinary and Food Safety Authority (ANSVSA), Focal Point in Romania for the European Food Safety Authority (EFSA), explained that in 99%+ of cases, there is a 4-5 day lag between identification of a presumptive positive and official confirmation and subsequent depopulation. In this narrow window, sample collection will be possible if done efficiently and without interfering with the government mandated depopulation. There is high motivation in Romania for collaboration so the expectation is that at least a subset of animals could be sampled and aliquots of collected samples shipped to FADDL for PCR testing.

Objectives:

- Receiving the samples from the team of veterinarians and verifying compliance of the transport criteria;
- Verifying the compliance of the samples with the sample identification protocol;
- Storing the samples in a specialized large capacity freezer at a specific temperature (-80 degrees Celsius);
- Sharing a report of the identification of the samples with the rest of the consortium after each batch of samples received;
- Preparing the stored samples (manipulation, packaging) for transport to the United States of America;
- Assisting in the expedition of the samples to the United States of America;
- Participate to the scientific publications.

Materials & Methods: The 3000 blood samples were collected from three farms where African swine fever (ASF) has been confirmed by the State Veterinary Animal Health Lab (African swine fever reference lab). The sampling was done as follows:

- Farm 1: 4-5 Aug 2021 (grower pigs): 1000 blood samples
- Farm 2: 16-17 Aug 2021 (finisher pigs): 1000 blood samples
- Farm 3: 3 Sep 2021 (finisher pigs): 1000 blood samples

Additionally, 197 saliva (oral fluids) samples were collected, as follows:

- Farm 1: 33 samples
- Farm 2: 100 samples
- Farm 3: 64 samples

Eight spleen tissue samples were also collected following necropsies:

- Farm 2: 7 samples
- Farm 3: 1 sample

All samples were transported from the site of sampling to the facilities of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca by our team and immediately frozen at v-80 C. All samples were stored in ultrafreezers until shipment, which took place on 5 October 2021. Prior to sampling, all samples were properly labelled and packed, and all required documents and forms were prepared and sent (part to USDA, part to World Courier).

Results: All samples transported, received, stored, packed and shipped according to the agreement.

Discussion: Not applicable.