# The use of PCR and Elisa methods to detect and monitor the infection of domestic pigs and wild boars with African Swine Fever virus

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#### Introduction

### Principle of PCR method

- Ensures the detection of African Swine Fever virus as long as it is present in the cell respectively in the state of viremia (the first or the second day after contact with the virus up to 3-4 weeks.
- DNA is extracted and purified.
- Selection of the specific sequence of ASF virus by specific primers.
- Amplifying the selected sequence and setting the CT (Threshold Cycle.

# Principle of ELISA method

- Detects antibodies that are produced by the body to fight the ASF virus.
- Antibodies are produced by the body from the 14th day after contact with the virus and can continue for several months to years.
- Wells are lined with recombinant proteins ASF (African Swine Fever) p32, p62, p72 Anti-ASF (African Swine Fever) antibodies if present form an antigen-antibody-conjugate-peroxidase complex The resulting staining is proportional to the amount of specific antibodies present in the presence of antibodies, a blue solution appears in the absence of antibodies no staining appears.

#### Materials and method

- This experiment was conducted in accordance to OIE manual for African Swine Fever.
- This experiment was conducted in accordance to European and national

- law's regarding the welfare and care of animals.
- For the collection of blood, 8 ml EDTA tubs were used for PCR test and for ELISA test 8 ml plain tubes were used.
- The organs and the bon marrow was collected from cadaver and these matrix were used only by the PCR method.

#### **Results and Discussion**

- Of the total wild boars confirmed with ASF using the Real Time PCR method and ELISA method during 2019.
- Using the PCR method we confirmed 28 wild boars cases of ASF.
- Using the ELISA method we confirmed 18 wild boars cases of ASF
- Only 6 wild boars cases were confirmed using both analyse methods this demonstrate the stage of infection (viremia), with clinical signs and in the period of seroconversion (> 10 days)
- Of the total domestic pigs confirmed with ASF using the Real Time PCR method and ELISA method during 2019.
- Using the PCR method we confirmed 15 domestic pig cases of ASF.
- Using the ELISA method we confirmed 5 cases of ASF.
- None of the cases were confirmed using both analyse methods this
  demonstrate that these animals has recently come into contact with the
  ASF virus ( < 7 days after contact) Clinical signs are not obvious.
  Antibodies are not produced.</li>

Table 1. Evolutionary dynamics of African swine fever in domestic pigs and wild boars in Constanta County 2019

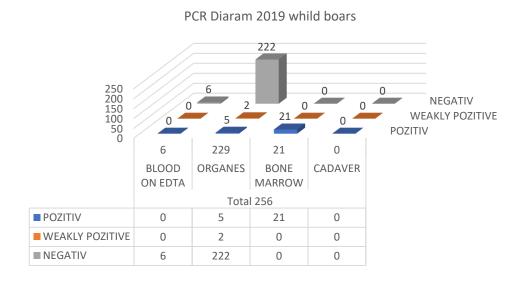


Table 2. Evolutionary dynamics of African swine fever in domestic pigs and wild boars in Constanta County 2019

PCR Diagram 219 domestic pigs from comercial holdings

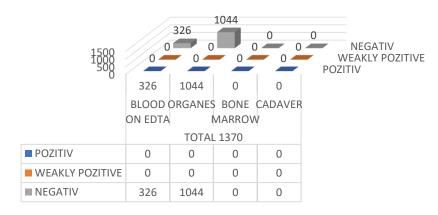


Table 3. Evolutionary dynamics of African swine fever in domestic pigs and wild boars in Constanta County 2019

PCR Diagram 2019 domestic pigs from private holdings

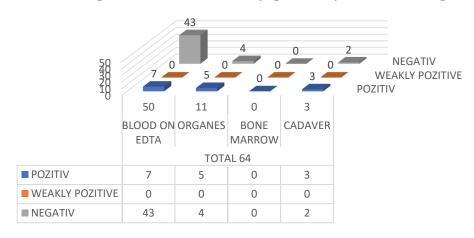


Table 4 Comparative testing of samples from wild boars

Diagram 2019 wild boar with pozitiv results using ELISA method in paralel with PCR method

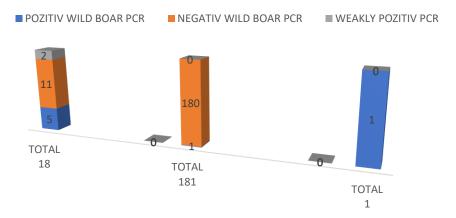
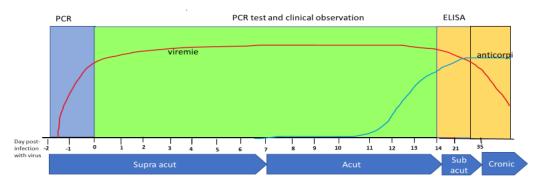


Table 5. The progress of the disease and the therapies used to identify the active infection

# The progress of the disease and the laboratory methods used to identify the active infection



- The objective of our study was to emphasize the sole purpose of conducting the PCR test that reveals the detection of the genome specific to African Swine Fever virus to determine the active infection.
- The PCR test is used in the case of animals showing clinical signs of African Swine Fever, or to determine if they are contagious.
- The PCR is a test that allows the identification of infected animals by obtaining the result of detected genome/ undetected genome.
- While the ELISA test is used to detect the presence of specific antibodies to African Swine Fever from the 14th day after contact with the virus.

- It is advised not to relay on the ELISA test to find out if the animals have recently been infected with the ASF virus
- The ELISA test is used in animals that do not show clinical signs of disease
- The ELISA can help statistically to keep a record of the percentage in terms of cases that have become immune to the disease.

## Reference

 $[1] http://asfreferencelab.info/asf/images/GUIDLINES\_Link\_3\_interpretation\_of\_results.pdf$ 

[2] Williams, D. (2021). African swine fever laboratory diagnostics GF-TADs African swine fever (ASF) Coordination Virtual meeting.









