African swine fever (ASF) is a low spreading viral infectious disease of pigs and wild boars. Other representatives of *Suidae* family are also susceptible. The most important vector of African swine fever virus (ASFV) in eastern Europe are wild boars. On the other hand in western Europe countries with a Mediterranean climate and Africa as well as some regions of Russia the ASFV vector may be represented by ticks belonging to *Ornithodoros* genus. ASF is a very important epidemiological and economical threat due to the limitations in international trade of pigs and processed products on the territory of the affected country. Despite the previous attempts no effective vaccine against ASF is accessible, what is caused by the unique features of ASFV. The conducted experimental studies on comparison of complete ASFV genomes of different strains indicate the importance of certain genomic regions in its virulence. The aim of this project is to investigate the structure and biological properties of ASFV recombinant strain lacking A238L, EP402R and 9GL genes. These genes have an influence on expression inhibition of genetic factors related to host immune response, hemadsorption as well as virus maturation. The study will be conducted on the representative ASFV strain selected using next generation sequencing analysis (NGS) from the collected pool of 93 isolates of the virus from wild boars and 3 isolates obtained from pigs. The previous studies conducted by the research team from the National Reference Laboratory (NRL) for ASF at the NVRI on the sequence analysis of A238L, EP153L, EP402R and MGF505-2R genes showed their 99.4 to 100% nucleotide sequence similarity to the Georgia 2007/1 strain. This strain has been introduced into the Georgia territory in 2007 and still circulates in wild boars and pigs in Russia, Lithuania, Latvia and Estonia. Construction of recombinant ASFV strain with introduced mutations within A238L, EP402R and 9GL genes may facilitate examination of the influence of induced mutations on the biological properties of ASFV in cell cultures as well as using pig model. The proposed research project has highly cognitive character and may aid in understanding and selection of possible gene candidates for further vaccine preparation.
The first task of the project aims the selection of ASFV strain for further construction of recombinant virus lacking A238L, EP402R and 9GL genes. The strain will be selected after next generation sequencing (NGS) analysis of 10 isolates from the collection of NRL for ASF at the NVRI. During the second stage of the project using CRISPR/Cas9 technology the inducted mutagenesis of A238L, EP402R and 9GL regions will be conducted. For this purpose, the guide (gRNA) fragments will be designed which are the matrix for Cas9 endonuclease associated with the CRISPR system (clustered, regularly interspaced short palindromic repeats). Precisely designed gRNA will allow cleavage of DNA fragments in A238L, EP402R and 9GL regions and then re-annealing of DNA-strands using homologous repairment system. The resulting recombinant strain of ASFV will be examined in VERO cell cultures or pig macrophage cell line. During the third research task the biological properties of constructed recombinant ASFV strain as well as parental strain will be investigated. These studies will be conducted on 30 pigs with determined immune status within the PCL3+ NVRI animal facilities with high biosecurity standards.
The proposed research has a great importance for the cognitive ASF epidemiology and immunology. The innovative CRISPR/Cas9 technology will be applied for the first time to obtain the recombinant strain of ASFV. This will allow feasible manipulation within selected ASFV genomic regions. The effect of this study may also be selection of possible candidates for further ASF vaccine preparation. The results of the research will be published in reputable journals focused on viral and infectious diseases of pigs. The results will be presented at the national and international conferences on pig diseases.