

The aim of the studies is to develop a robust and low-cost surveillance protocol for influenza A virus (IAV) in swine which will allow to easier update of our knowledge on virus' epidemiology and genetic diversity. Influenza plays significant role as a primary and secondary pathogen of respiratory tract having negative impact on pig production. We will focus also on a less known aspect of pig influenza, that is its ability to persist on farms and cause less dramatic clinical outcome as in case of infection in naïve populations. Non apparent, sometimes subclinical infections of pig herds, involving multiple IAV subtypes, in the face of partial cross protection between them, pose a risk of emergence of new variants of yet unknown epizootic and epidemic potential. As the efficacy of the active surveillance testing nasal swabs to monitor the IAV evolution in pigs is compromised by difficulties to correctly diagnose the disease clinically, very mild or subclinical course in some cases and short period of IAV shedding, we propose to evaluate the applicability of oral fluid testing for the detection and passive surveillance and subtyping of IAV. The objectives of the project are evaluation of applicability of oral fluid testing for the diagnosis and subtyping of IAV infections, evaluation of IAV circulation patterns and genetic diversity on Polish farms of different size and production systems. The project consists of four major tasks. In task 1 several farms infected with IAV will be visited and sampled. RNA will be extracted and real time RT-PCR for general detection of IAV will be performed. Next, the positive samples will be analysed in multiplex real time PCR to subtype IAV. This will allow to compare the sensitivity of IAV detection and typing in these two materials. From selected samples with low Ct values complete genomic segments will be amplified. The segment amplicons and cDNA from selected clinical samples (e.g. $Ct \leq 20$) will be sequenced with Next Generation technology. Guidelines for selecting an optimal workflow for IAV sequencing from nasal swabs and oral fluid samples will be developed. In task 2 about 60 farms will be visited and nasal swabs and oral fluids will be collected from different age groups. From a farrow to finish farms at least 35 swabs and 7 oral fluid samples will be collected. The samples will be tested with real time PCR as described above. This will allow for evaluation of prevalence and circulation of various IAV subtypes in farms of different production type. In task 3 selected positive samples will be sequenced according to guidelines developed before. In the final task 4 we will analyse genetic diversity of Polish IAV strains from pig farms of different size, geographic location, production types, sampling season and IAV vaccination status and nucleotide sequences of IAV strains from Poland will be benchmarked with current data from Denmark, Germany, France, Spain and Italy. The outcome of the project will be the development of efficient and low-cost protocols for IAV detection and subtyping using real time PCR and oral fluid of pigs. Testing many farms for the presence of IAV will provide material for sequencing of all virus genome segments. Their analysis will allow for unprecedented analysis of genetic diversity of IAV in Polish pigs what is important for European epidemiology of this virus as well. Moreover, the role of international live pigs movement in IAV evolution will be assessed.