

Avian influenza virus (AIV) is one of the most important poultry pathogens. Based on the clinical disease, two pathotypes of AIV are distinguished: low pathogenic (LPAIV) and highly pathogenic (HPAIV). Highly pathogenic H5 subtypes pose a significant problem for poultry industry, H7 subtypes also cause occasional outbreaks of HPAI. Additionally, some subtypes of AIV (e.g. H5N1, H7N9, H10N8) are capable of infecting humans.

As other RNA viruses, AIV is also characterized by high genetic variability, which results in generation of diverse pool of virus variants related to the dominant lineage during replication in infected host. The host and environmental conditions determine survival of emergent mutants, therefore the appearance of variant possessing an advantageous mutation can lead to selection of this variant and change in the dominant lineage in virus population. The extent of viral population diversity is of great significance for the virus adaptability, because more heterogeneous viral populations are more capable of adaptation in a dynamically changing environment. The investigation of presence and contribution of minority variants in virus population is important for studies on virus evolution, early detection of mutants with increased pathogenicity or potential to cross host-species barriers. Thanks to the next generation sequencing (NGS) methods studying the viral population diversity has become more feasible and reliable and can be also applied in research on the evolution of influenza virus. Such studies have been performed for human influenza virus or avian influenza dangerous for humans (e.g. H5N1, H7N9) in mammalian species.

There are only few studies regarding the diversity of AIV in poultry, mainly based on clinical samples from field outbreaks. However, none of them addresses the question of dynamics of changes in virus diversity during the course of infection in the same host, or how the diversity changes following transmission to contact birds. The proposed project provides experiments to analyze the virus diversity in the course of infection in two most prevalent poultry species – chickens and turkeys. For this purpose five birds of each species will be infected with H7N7 AIV, five birds will have direct contact and five another will have indirect contact with infected birds. At predetermined time intervals swabs will be collected from all birds and then will be subjected to NGS to determine the presence and contribution of individual variants in each sample. The analysis will provide information on the differences in AIV diversity in both species, which will help to determine their differing role in AIV evolution. Turkeys are more susceptible to AIV infection than chickens and are considered as favorable environment for virus adaptation, and the study will give answer if this phenomenon is associated with increased virus diversity in this species. Additionally, evaluation of virus diversity in contact birds will elucidate the impact of bottleneck effect (a phenomenon of decrease in virus genetic variation), which is a factor causing limited virus fitness or loss of beneficial mutations.

Another experiments will involve generation of mutant H5N1 virus possessing a polybasic cleavage site in hemagglutinin, which is a main determinant of pathogenicity. Then chickens will be infected with mixtures containing different amount of this mutant and contact birds will be co-housed with infected chickens. Samples collected from birds will be subjected to NGS to see how the initial proportion of virulent variant and transmission bottleneck affect the rate of its selection during virus replication.