H9N2 avian influenza virus (AIV) is an important pathogen from both economic and epidemiological point of view. It has become endemic in poultry in Asia and Africa, i.e. is being found persistently in certain regions of these continent, where in combination with other pathogens may cause significant losses in the poultry industry. Because of its preference to specifically bind to receptors in human airways it is sometimes capable of infecting humans (it has "zoonotic potential") and poses a threat to public health. A novel variant of H9N2 virus emerged in Europe in 2012, first outbreaks were reported in turkeys in Germany. Over the next two years it had been also detected in fattening turkey flocks in Poland. The analysis of relationship showed that the virus did not originate from poultry in Asia or Africa, but its source were probably wild birds of Eurasia. Detection of outbreaks year after year and identification of signatures of adaptation to poultry in the viral nucleic acid indicate that the virus has established itself in the poultry population. Moreover, the analysis also revealed the emergence of genetic changes indicative of a dynamic nature of the current epidemic, i.e. in one of the isolates a mutation in the PA gene responsible for the increased efficiency of virus propagation in mammalian cells was detected.

Due to the economic losses caused by H9N2 virus in Asia and its possible zoonotic potential, it is necessary to gather more information about the pathogenicity of this new variant, which is the goal of this project. The research provides evaluation of pathobiology of infections with the novel variant of H9N2 AIV by experimental infection of specific-pathogen free (SPF) turkeys, and commercial quail and ducks and assessment of clinical signs, level and duration of virus shedding, virus transmission potential as well as development of immune response measured as the level of specific antibodies in sera. Preliminary tests in turkeys infected with other respiratory bacterial pathogens (Ornithobacterium rhinotracheale, Bordetella avium), demonstrated that co-infection with H9N2 virus can trigger severe course of disease. Therefore, for the objective assessment of the pathogenic potential of H9N2 virus for these birds which are the main sensitive species, the use of specific pathogen free (SPF) turkeys (i.e. housed in isolation and free from any infections with pathogens) is highly desirable. It would help answer the question, whether the virus itself can cause disease and mortality, and show the efficiency to be passed on to contact birds, thus clarifying whether the infection can pass unnoticed, enabling the virus to spread further and evolve. This is important due to the fact that turkeys have been found to be more sensitive to low pathogenic AIVs than chickens and can act as an intermediate host between wild birds and other poultry species, providing a favorable environment for an adaptation process. Since the novel H9N2 virus has already shown markers of adaptation to poultry, its transmission to other domestic birds seems probable. Therefore, the project also foresees experiments on other poultry species, i.e. quail and ducks. Experimental infection of quail will elucidate whether the current level of adaptation of the new variant is sufficient to induce disease in these birds and if they can be a silent reservoir (i.e. exrete the virus without showing symptoms). The same problems would be solved in case of ducks, representatives of waterfowl, which are a natural reservoir for low pathogenic avian influenza (i.e. in which the virus multiplies without causing any significant damage but making a source of infection to susceptible birds). Therefore they play the major role in the epidemiology of avian influenza, especially that domestic ducks are often reared in free–range farms sharing habitat with their wild relatives. The second series of experiments is aimed at the evaluation of changes emerging in the genetic material of the H9N2 virus as a result of passages in chickens (consecutive infections of groups of chickens where the virus grown in one group of birds is passed on to another group and the procedure is repeated 10 times). For this purpose, two viruses will be used – one derived from wild birds, not adapted to poultry and one from turkeys, showing changes indicative of adaptation to poultry. Ten passages of each virus will be carried out and isolate from each passage will be sequenced in order to pin down the primary structure of the nucleic acid to track mutations in viral RNA, especially those associated with adaptation, enhanced pathogenicity or increased zoonotic potential. The results will contribute to the understanding of mechanisms enabling adaptation of AIVs to chickens, which are the most prevalent poultry species worldwide and thus the most important one in AIV transmission cycles in poultry populations. It is well known fact, that under natural conditions mutations arise as a result of constant passages of the virus so the monitoring of this phenomenon in controlled laboratory conditions will allow for better understanding of mechanisms underlying the great genetic variability of flu viruses.