Summary

Colibacillosis, as a common bacterial disease causing high mortality among poultry, is a significant epidemiologic problem contributing to large economic losses for breeders. The disease occurs as a result of infection with Avian Pathogenic *E. coli* (APEC). These strains are classified as extraintestinal pathogens (ExPEC), which carry virulence factors important for the pathogenesis, such as: adhesins, toxins, protectins, or factors related to the iron uptake from the environment. The proper diagnosis of colibacillosis is extremely important for disease prevention in flocks, application of targeted therapy and microbiological safety of food. At the same time, the identification of the strains responsible for the infection is very difficult due to the fact that APEC constitute a heterogeneous group with a multifactorial mechanism of pathogenesis. In addition, the spread of commensal *E. coli* in the environment causes problems in isolation of strains and assessing their pathogenicity, as these strains can also cause infection under certain conditions. Therefore, it is extremely important to develop a diagnostic method that will quickly and unambiguously identify pathogenic strains causing infection in flocks.

The main goal of the presented thesis was to develop a diagnostic method based on PCR reaction that will allow for the identification of Avian Pathogenic *E. coli* (APEC). For this purpose, a collection of strains from birds affected by colibacillosis as well as from healthy birds was collected. Then the strains were differentiated, their genomes were sequenced and a bioinformatic analysis was carried out to select the appropriate virulence factors responsible for causing colibacillosis. The diagnostic test, developed using *in silico* method, was verified by laboratory methods.

Differentiation of *E. coli* strains was performed based on the MP PCR method, analyzing the resulting band profiles in the BioNumerics software. It allowed to reject identical strains from further studies of and reduce the number of samples subjected to sequencing. Next Generation Sequencing (NGS) of whole genomes (WGS) was performed on the Illumina platform and genome assemblies were prepared in the SPAdes software with the necessary manual editing. The obtained results allowed to carry out a series of *in silico* analyzes: the assessment of the presence of selected virulence factors, serotyping or the presence of antibiotic resistance genes. Additionally, PCR analysis of the phylogenetic groups of the studied *E. coli* strains was performed according to the method of Clermont et al., (2013).

In order to be able to construct a correct model for predicting the pathogenicity of *E. coli* strains, they were reclassified from the original division based on the strains' source to the one based on the results of studies in the *in ovo* model. The obtained results allowed to divide the strains into two groups (83 pathogenic strains and 17 non-pathogenic strains) and to select such virulence factors that correctly differentiate bacteria into these two groups.

The diagnostic test was designed based on the multiplex PCR amplification of 2 virulence genes (*iroC* and *hlyF*) and the gene encoding the O-antigen flipase in O78 strains. According to the designed method, the presence of any of these genes indicates that the strain belongs to the group of pathogens, otherwise the sample is a commensal strain. During the verification of the results from the model in relation to the chicken embryo model, the method accuracy was 93.00% and the sensitivity and specificity equal to 98.80% and 64.71%, respectively. The shift of the cut-off point towards sensitivity results from the fact that quick identification of a sick flock and the possibility of implementing treatment was a priority during the research. The method was optimized and a 100% agreement of the laboratory results with the *in silico* results was obtained.

In conclusion, the use of whole genome sequencing of *E. coli* allows to develop a quick diagnostic method to identify pathogenic strains for poultry: APEC. The developed test, based on PCR, together with the isolation site of strains and clinical symptoms of the disease, can be used in practice after validation.