The main goal of our project is to examine the molecular mechanisms of pathogenesis of *E. coli strains,* which have an extraintestinal pathogenic potential (ExPEc, extraintestinal pathogenic *E. coli*) both with *in vitro* and *in vivo* methods. Essential in this research is an analysis of the virulence factors that are crucial in the survival of E. coli in the hostile serum environment.

Our project includes the following research objectives:
a. Determination of the virulence genes (related to pathogenicity of ExPEC including genes of increased survival in host serum) profiles of the tested *E. coli* strains and examination of phylogenetic relationships between the examined bacterial strains.
b.The *in vitro* tesiting of the pathogenicity of tested *E. coli* strains by the determination of the resistance to bactericidal activity of host serum.
c. The *in vivo* tesiting of the pathogenicity of tested *E. coli* strains by the observing the changes in the survival of larvae of *Galleria mellonella*.
d. Examination of potential changes in the expression of the selected virulence genes in response to exposition of tested *E. coli* strains to bactericidal activity of serum.
e. Examination of the changes in outer membrane proteome of the tested *E. coli* strains after exposure to serum and identification of potential OMPs (Outer Membrane Proteins) responsible for the resistance of extraintestinal pathogenic *E. coli* isolates to bactericidal activity of serum.

Escherichia coli remains one of the best–known, best-understood and best-studied microorganisms that have been used as model organisms in a lot of research in the field of cell metabolism, genetic engineering or other investigations. *E. coli* is characteriz ed by huge genetic diversity and abilities to occupy a wide range of ecological niches. A commensal *E. coli* strain constitutes a predominant compound of intestinal microflora of warm-blooded animals including humans. However *E. coli* species also includes pathogenic strains which have specific virulence factors conditioning the abilities of these bacteria to develop an infection . In public opinion *E. coli* is mainly considered as an etiological factor of gastroenteric infections. Diarrheagenic *E. coli* (DEC) group of pathogens divides into six pathotypes endowed with a special combination of virulence traits (VG) resulting in a unique diarrheal syndrome with diverse outcome and sensitivity. It is uncommon that diarrheagenic E. coli strains can be a constituent of intestine microflora. If these bacteria emerge in host organisms they will cause colitis or gastroenteritis but almost never extraintestinal infection. Currently one of the most compelling problems of public health worldwide is enterohemorrhagic *E. coli* (EHEC) that can develop a severe disease, including bloody diarrhoea, often complicated by a haemolytic uremic syndrome (HUS), which leads to kidney failure . But there is another group of pathogenic *E. coli* such as extraintestinal pathogenic *Escherichia coli,* referenced as ExPEC, that is often not perceived and even diregarded as a serious health risk.
Extraintestinal pathogenic *E. coli* (ExPEC) are able to establish an infection in almost every site of the host body outside the gastrointestinal tract including the urinary tract, the central nervous system, the circulatory as well as the respiratory system. ExPEC have abilities to cause a wide range of diverse infections with a severe outcome. ExPEC include the following *E. coli* strains isolated from infections outside the intestinal tract of host: uropathogenic *E. coli* (UPEC), neonatal-meningitis *E. coli* (NMEC), sepsis-causing *E. coli* (SEPEC) and avian pathogenic *E. coli* (APEC) causing colibacilliosis in poultry as well as in wildfowl.
It has been proved by phylogenetic analysis that extraintestinal *E. coli* evolved from commensal *E. coli* strains by the acquisition of virulence genetic factors via horizontal transfer. It has been revealed in studies that ExPEC strains were isolated as a component of intestine microflora in 20% of the tested healthy people. Frequently, the colonisation and protective function of ExPEC is even more effective than the typical non-pathogenic commensal *E. coli*. Although ExPEC actually associate with the host as commensal microflora, they will cause a disease if they get out of the intestine into a normally sterile body site.
ExPEC strains are associated with infections in a variety of animals, but *E. coli* strains pathogenic to humans and poultry have the greatest epidemiological meaning . Uropathogenic strtains of *E. coli* is the main etiological factor of urinary tract infections (UTIs) in humans. Annually, there are about 130–175 million UTIs (usually caused by UPEC strains) diagnosed worldwide. It is estimated that up to 60% of women have at least one episode of UTI and mostlx with uropathogenic strains of *E. coli* as etiologial factor, in their lifetime. But what is most important in terms of health –public risk, UPEC are able to cross the epithelial cells of the urinary tract and gain access to the bloodstream, causing bacteremia and sepsis refered as urosepsis. It is estiamted that urosepsis comprises about 25 % of all sepsis cases and in most cases is due to complicated urinary tract infections. In a recent study of bacteremic UTIs, *E. coli* was the most common pathogen, responsible for nearly 75% of all bacteremia caused by gram-negative. UPEC are abe to establish infection not only in urinary tract but also in any site of host organism, because of that uropathogenic *E.coli* are included in the recently defined pathotype ExPEC.
In the presented project we would like to use the determination of prevalence of selectec virulence gens to classification and characterisation of tested *E. coli* strains. We have also planned to analyse a molecular mechanisms of pathogenesis of tested *E. coli* strains both *in vitro* as well as *in vivo* conditions. In this aim we would like to perfome a passages of tested strains in human serum and also assessment of virulence of bacterial isolates on inscet model of *Galleria mellonella*. Larvae of *G. mellonella* have an application as a model organbism to investigate the virulence mechanisms of many pathogens such as enteropathogenic *E. coli* (EPEC) or uropathogenic *E. coli* (UPEC). But also, *E. coli* isolated from animal sources i.e. avian pathogenic *E . coli* (APEC) or *E. coli* isolated from reptilesm feces have not been tested on this insect model so far, this experiment will be a great innovation in presented project. An essential and promissing in presented study is examination of changes both in transcriptome (changes in selected genes expresion) and as well as in outter mambrane proteome of tested *E. coli* strains in response to exposition to bactericidal activity of serum.

The innovative aspect of this project is the characteristic and analysis of *E. coli* isolated from reptiles representing cold-blooded animals. This part of the study seems to be novel and interesting because, as it has been mentioned above, there is little information available about cold-blooded animals as a reservoir of *E. coli*. ExPEC are widely disseminated in the environment and can be isolated from livestock, i.e. poultry, cattle, swine, as well as from pets (dogs, cats, horses) and wild animals. But the knowledge about prevalence of *E. coli* among cold-blooded animals is scanty.

The studies published so far have only included the isolation of *E. coli* from reptiles' feces and the phenotypic characteristic of these isolates such as determination of antibiotic resistance patterns and the assessment of biochemical properties. However, there is no study showing an analysis of *E. coli* isolates of cold-blooded animals in terms of virulence factors, zoonotic potential of these isolates such as abilities to survive in serum of warm-blooded animals and assessment of pathogenicity of these strains both *in vivo* and *in vitro*, which is the main aim of the presented project. Reptiles frequently carry a pathogenic for human bacteria i.e. *Salmonella* that can cause serious illness in people, called reptile-associated salmonellosis, RAS. Because of that reptiles could be a hipothetical carriers of other bacterial pathogens like as extraintestinal pathogenic *E. coli.* In presented project we would like to perform a molecular analysis of *E. coli* isolated from reptiles in comparission to APEC and UPEC strains which will include a vitotyping in terms of virulence genes, analysis of outter membrane proteome aand determination the virulence *in vitro* and *in vivo.*

The presented project includes the analysis of the prevalence in the environment of an important virulence factor, i.e. resistance of *E. coli* rods to bactericidal serum activity. We have also planned to compare UPEC, APEC and commensal strain *E. coli* isolated from healthy reptiles in terms of virulence factor and abilities to survive in the serum environment. The desired results of this study are determination and identification of genetic factors and outer membrane protein responsible for serum resistance of all pathotypes of extraintestinal pathogenic *E. coli.*