Zoonoses are one of the main problems of healthcare worldwide. WHO estimated that 60% of human infectious diseases are zoonosis. Reptiles are a source of gram-negative bacteria, mainly *Salmonella* spp., that can cause a zoonoses ***Salmonella*** rods are one of the leading causes of humans intestine infection in Europe and the world. More than 2,600 *Salmonella* serological variants have been described. *Salmonella* rods are pathogenic bacteria for humans, but animals may be asymptomatic carriers. All of the *Salmonella enterica* subspecies are isolated from birds, rodents, dogs, cats and reptiles. *Salmonella enterica* subsp. *arizonae* and *diarizonae* which are are characteristic for reptiles and rarely isolated from humans. *Salmonella* strains isolated from reptiles can cause infections calls RAS (Reptile-associated salmonellosis), which can be serious infection for risk groups as children, and elderly mainly. RAS infection can occur through direct or intermediary contact with a reptile. This is possible due to the ability of *Salmonella* to produce biofilm. Biofilm is a complex structure which is consist of bacterial cells adhering to the surface and an extracellular matrix and allows to survive in the external environment outside the host organism. Among *Salmonella* spp. isolated from reptiles, variants that are resistant to the human complement system and capable of multiplying in human blood serum are the most important.

The reason for research proposed in this project is the limited availability of literature data about microbiota of free-living snakes and reptiles in Poland as well as a small amount of a data about biofilm forming and lack of data about induction of innate immune response by rarely isolated serovars characteristic for reptiles.

The main goal of the project is to characterize virulence factors of rare serovars of *Salmonella* spp isolated from snakes living wild in Poland. Bacteria from grass snake (*Natrix natrix*), dice snake (*N. tessellata*), smooth snake (*Coronella austriaca*), and Aesculapian snake (*Zamenis longissimus*) will be tested. The preliminary studies have indicated the biofilm production under static conditions and the influence of nutrient availability and temperature on this process. In addition, the ability of the analyzed strains to multiply in the presence of human blood serum was demonstrated, which confirmed resistance to the human complement system.

The planned project tasks will consist of assessing the ability to produce biofilm under microfluidic conditions, identifying the components of the biofilm matrix and the activity of genes involved in this process, detecting and determining the expression of virulence genes. Gene expression and biofilm production will be tested under varying temperature conditions and nutrient availability. Virulence assessment will also be tested on the *Galleria mellonella* in vivo model. The larvae of this insect will be infected with bacteria and their response to infection will be monitored. In addition, non-specific immune defense proteins produced in response to infection will be isolated and identified from surviving larvae. Chosen genomes of strains differing in pathogenicity and ability to produce biofilm will be fully sequenced.

Anticipated effects include confirming reptiles as a source of biofilm-producing bacteria with zoonotic potential, and indicating genetic variability among rare serological variants of *Salmonella* spp. A expected outcome is the confirmation that both gene expression and biofilm formation are temperature and nutrient-dependent processes, and their decrease will promote specific gene expression and the biofilm phenotype of isolates. Additionally, the influence of these conditions on larval survival and the nonspecific immune response is also expected.

The scientific objectives of the project may have practical implications in everyday life. The results obtained during the project could be used by other researchers, as well as biologists, educators, veterinarians, and reptile breeders. Tested snakes are not endemic in Poland but also living in other countries, therefore the obtained results are not only of local importance