ASSESSMENT OF VIRULENCE POTENTIAL OF *PROTOTHECA* MICROALGAE, OPPORTUNISTIC PATHOGENS OF HUMANS AND ANIMALS – *IN VITRO* AND *IN VIVO* STUDIES

The genus *Prototheca* comprises unicellular, achlorophyllous microalgae, ubiquitously distributed in the environment. Five of the eight currently known species, namely *P. zopfii*, *P. wickerhamii*, *P. blaschkeae*, *P.cutis*, and *P. miyajii* have been implicated in opportunistic infections, collectively referred to as protothecosis. *Prototheca* spp. are the only known plants that have repeatedly been reported to infect vertebrates, including humans. The most common form of animal protothecosis is bovine mastitis, which represents a serious veterinary problem and incurs heavy economic losses to the dairy industry worldwide due to reduced milk production, increased costs of veterinary treatment, and early culling. *Prototheca* spp. are emerging pathogens that pose a serious threat also to human health. At particular risk are patients suffering from different forms of immunosuppression. A common resistance of *Prototheca* spp. to conventional antimicrobial therapies currently employed in medicine results in poor treatment outcomes and contributes to a more severe course of the disease.

Prototheca algae and protothecosis in general have been much neglected areas of research. However, an apparent rise in the number of cases of human and animal protothecosis over the last decade globally and the biological uniqueness of *Prototheca* spp. in the entire Plantae kingdom have recently brought more attention to those microorganisms. The pathogenesis of *Prototheca* spp. infections has been poorly addressed, leaving routes of entry, predisposing factors, and host-pathogen interactions almost unexplored. There have been no studies that would have, thoroughly and comparatively, investigated the pathogenicity of different *Prototheca* species and genotypes. Also, very little is known about virulence factors of *Prototheca* algae. The lack of established *in vitro* and *in vivo* experimental models of protothecosis has seriously impeded the exploration of virulence mechanisms in Protothecans. Thus, the purpose of this project is to determine the ability of different *Prototheca* species to induce local or systemic infections and to examine host-pathogen interactions both *in vivo*, in an animal model, and *in vitro*, using a set of eukaryotic cell lines.

The project will employ a bidirectional approach, combining *in vitro* studies, with different mammalian cell lines, and *in vivo* murine experimental model. A protocol for the preparation of infectious doses will be developed. Specific *in vitro* assays will allow adherence, phagocytosis, and survival of the algal cells within the eukaryotic cells, including epithelial cells and macrophages, to be assessed. Determination of the localization of the pathogen in relation to eukaryotic cell (intra- and extracellular) will be of particular importance and this will be achieved, in parallel, using antibiotic resistance assays and microscopic analysis of immunofluorescently labeled tissues. The viability of mammalian cells before and after infection will be analyzed with the fluorescent assays. Both immunocompetent and immunocompromised mice will be used for the animal model. The animals will be inoculated with different *Prototheca* species (genotypes), via different routes, and using different challenging doses. Peripheral blood will be collected, at designated time points, for standard haematological and biochemical examinations, and to assess circulating immune cell subsets and levels of inflammatory cytokines. Post-infection mice will be euthanized and their organs will be processed for histopathology and *Prototheca* culturing, so that the algal yield in tissues can be measured.
The data obtained during the project will be subjected to multiple comparisons and extensive statistical analyses in order to develop a complete infection model of animal protothecosis.

The research project, under this proposal, is conceived as the most extensive study ever performed on the pathogenesis of protothecosis. The results to be obtained will substantially expand the current knowledge on the virulence of *Prototheca* spp., shedding important light on the impact of these microorganisms on human and animal health. The outcomes of the project are believed to pave the way for the development and implementation of new diagnostic assays allowing for an accurate determination of virulence and infectivity of clinical isolates. Finally, the results will bring us much closer to the development of new, effective methods of treatment and prevention of human and animal protothecosis.