ABSTRACT

The work was focused on the study of various aspects of development of *Leishmania* in vectors of genus *Phlebotomus* and can be divided into two main parts.

In the first part, we studied the competition of *Leishmania major* and *L. turanica* during their development in the sand flies who are natural vectors of *L. major* using the experimental co-infections of fluorescently marked promastigots of these two species. While both leishmania species developed similar in the intestine of *Phlebotomus papatasi*, *L. turanica* prevailed in *P. duboscqi* in the late stages of infection. The fluorescent marking of Leishmania should allow us also to study possible genetic exchange between species at different stages of *Leishmania* infection (2nd, 9th and 14th day after the infective feeding). Using the flow cytometry (FACS) we have repeatedly identified dozens of objects emitting red and green signals in the intestinal homogenates of co-infected sand flies, however further analysis with confocal microscope disproved these objects as the hybrid promastigots of *Leishmania*.

In the second part of this thesis we investigated the role of *L. major* genes HASP and SHERP, which lies on the locus LmcDNA16 and are expressed exclusively in metacyclics. We used mutant lines KO (lacking the locus LmcDNA16) and HASPB (KO line with gene HASPB back inserted) and we compared their development in the vectors and the metacyclogenesis to the FVI control lines. It was also important to determine whether the mutant lines are capable be transmitted to mice by infected sand flies. *Phlebotomus papatasi* infected with FVI control line transmitted the parasites into the mouse in 7% of the cases, while HASPB line was transmitted in 4% and KO line in 3%. Considerable differences were observed using the vector *P. duboscqi* where FVI control line was transferred in 20%, while the KO line in 7% and HASPB line transfer was completely negative. Measurement of morphological forms in the intestinal smears and qPCR showed that both mutant lines form metacyclic stages a smaller degree and are propagated less than FVI line in late-stages of infection. We believe that both of these differences can cause a reduced ability of transmission of the mutant lines by the infected sand flies .

Key words: *Phlebotomus*, *Leishmania*, HASP, SHERP, metacyclogenesis, genetic exchange