SUMMMARY

Title: „Molecular biological methods in laboratory diagnosis of pathogenic leptospires“

During Ph.D. study programe real-time PCR method based on detection of gene encoding surface lipoprotein LipL32 was designed, devised and introduced into clinical laboratory practice of laboratory diagnosis of acute form of leptospirosis. Positive and negative analytical specificity 100 % in both cases was defined and limit of detection in range 1 – 5 copies of genome / ml of liquid biological material was determined. Real-time PCR method was in clinical practice on biological materials gained in period April 2010 – April 2013 from 295 patients suspicious on leptospirosis verified. Total number of 9 persons from whom 15 biological materials originated as LipL32 positive were evaluated.

Real-time PCR was tested during the analysis of environmental samples for the presence of *Leptospira.* From 680 samples (surface water, waste water, wet substrates) were 5 real-time PCR reactions (0.7%) evaluated as borderline, results sequence analysis were repeatedly "uncultivable bacteria." All samples were as negative evaluated. From mention is clear that this method is not suitable for testing these materials.

Multilocus sequence typing analysis detecting 5 genes (*adk*, i*cdA*, *rrs2, lipl41* and *lipl32* gene) was used to create the library of sequences of 11 laboratory strains of pathogenic *Leptospira*. With its help could then be determined which infecting serovar of pathogenic leptospires in patients with suspected leptospirosis infection was present.

In retrospective study of leptospirosis in the Czech Republic in the years 2002 - 2013 total of 5840 patients with suspected leptospirosis were examined, from whom 101 persons (1.7%) were evaluated as LipL32 positive. The disease was diagnosed more often in men (n = 71, 70.3%) than in women (n = 30, 29.7%). Maximum incidence of leptospirosis was recorded in 2002 (total 21 persons) and 2005 (total 25 persons), when the Czech Republic was affected both large and local flooding. The most frequent infectious type in our study was serovar *Leptospira grippotyphosa* (n = 43; 42,6 %).

To confirm the results of real-time PCR analysis the results of microagglutination test and ELISA were evaluated. Total of 1553 members of the Army of the Czech Republic were using ELISA method for the presence of specific antibodies IgM and IgG examined. In case of IgM 292 (18.8%) and IgG antibodies 392 (25.5%) persons as leptospira-positive were determined. The vast majority of these positive blood serum was using microagglutination test with 11 laboratory strains of pathogenic *Leptospira* used in the Czech Republic and non-pathogenic *Leptospira biflexa* as negative evaluated. In 26 blood sera (1.7%) was recorded weak reactions in titers 1:50 - 1:200.