Summary

Hepatitis A virus (HAV), an RNA virus, is a common cause of acute hepatitis that affects millions of people worldwide. The virus is transmitted through the fecal-oral route via person-to-person contact or through contaminated food or water.

During last decade several HAV outbreaks occurred in European countries, the latest HAV outbreak in Estonia was reported in 2011.

A set of molecular epidemiological methods were used for HAV outbreaks investigation, including amplification of genomic fragment, sequencing analysis and selection of genomic region used for phylogenetic analysis.

The aims of the present thesis were to learn general methods of molecular typing for outbreaks investigation, specifically to build a complete HAV genomes database and to align retrieved sequences, to identify conservative genomic regions and to perform phylogenetic analysis for created dataset, to compare performance of two HAV RNA extraction methods and to setup sequencing reaction for amplified fragments.

For these purposes, a complete HAV genomes database containing 56 sequences was built and retrieved sequences were aligned using ClustalW algorithm. In the result, 29 strains belonged to genotype 1A, 5 strains to genotype IB, 17 strains to genotype IIIA, 3 to genotype IIIB, 1 to genotype IIA and 1 to genotype IIB. Sequences alignment and phylogenetic tree construction were performed using BioEdit and Mega programs.

To identify variable genomic regions of sequences, values of nucleotide pairwise distance were calculated in SSE program. As the result, regions with p-values ranged 0,122–0,149 proved to be potentially suitable for phylogenetic analysis, including outbreak investigation.

To compare two HAV RNA extraction methods, 20 HAV-IgM positive human serum samples were used for setting up of extraction method, PCR reaction and sequencing of HAV RNA within VP1/2A genomic region. HAV RNA extraction was performed by two different methods:

by using TriPure Isolation reagent and QIAamp RNA Mini Kit. Independently of extraction method, HAV RNA could be amplified in 19 out of 20 (95%) HAV IgM positive serum samples. Because of similarity in results, determination of the end detection point of HAV RNA was performed to compare relative performance and robustness of two extraction methods. According to the results, HAV RNA yield was 10–1000-fold higher using QIAamp RNA Mini Kit, that demonstrates improved sensitivity of this method.

PCR reaction and sequencing of HAV RNA within VP1/2A genomic region were performed with two randomly selected amplified fragments, using primers recommended by RIVM protocol. It was possible to sequence strains belonging to genotype IB and IIIA.

Thus, it was prepared ready to use protocol for HAV outbreak investigation. It was also shown how bioinformatics tools could be applied to select genomic region for phylogenetic analysis.