Summary

This thesis describes the determination of the relationships between of 25 bovine non-O157:H7 Shiga toxin-producing *Escherichia coli* (STEC) strains by multilocus sequence typing (MLST) and compared with an O157:H7 strain and the *E. coli* K12 reference strain MG1655. For the phyologenetic analyses the four housekeeping genes *cadB* (442 bp), *mdh* (810 bp), *putP* (483 bp) and *trpC* (420 bp) were subjected to partial sequence analysis. Genes of the locus of enterocyte effacement (LEE) acquired by horizontal gene transfer, i.e. the highly variable virulence genes *eae* (895 bp) and *espB* (275 bp) were also analysed. Cladograms were established on the basis of the data obtained. The reliability of the cladograms was visualised by means of a combined analysis in a maximum parsimony tree (MPT). The aim of the investigations was to trace the possible developmental history of these LEE-positive bovine STEC strains.

The analysis of the housekeeping genes revealed the presence of possibly six different clusters. Sequence analysis of further housekeeping genes and a larger amount of data would have been necessary to arrive a final determination of the clusters. As regards the virulence genes, the phylogenetic data on the *esp*B were more or less the same as those of the *eae* gene, which can be taken as an indication of their co-evolution. There was also an absolute association between the 8 different types of intimin identified and the phylogenetic relationships between the strains. Intimin can thus be considered to be a phylogenetic marker.

The phylogenetic development of the strains as established by the analysis of the housekeeping genes was also compared with the data on the LEE-associated genes, in order to be able to estimate the time at which the LEE may have been transferred. Owing to the small number of genes investingated it was not possible to arrive at a final hypothesis, however, from the data it would appear that the LEEs were inserted several times in different *E. coli* phylotypes, independently of each other. The further development of the respective LEEs took place following integration of the LEE in the respective *E. coli* chromosomes and they were then analysed separately for the genes *eae* and *esp*B. The differences between their developments are clearly reflected in the pronounced differences in sequence size and sequence order. Thus, they revealed the formation of clusters of the housekeeping genes which must have developed as a result of different mutations over time. This led to the conclusion that there are at least five, probably even six different clusters which only partly

confirm the serotypes established by Wittam (Whittam, 1998) and Reid (Reid et al., 1999; Reid et al., 2000) the aid of MLEE (Selander and Lewin, 1980; Selander et al., 1986). These clusters are also reflected in the virulence genes and it can thus be assumed that each strain inserts an LEE independently of the others, but that its development takes a different course from this time onwards. The most pronounced changes revealed in the zeta cluster may have been attributable to a relatively higher selection pressure or have come about purely because they needed longer to develop, i.e. were simply older. More comprehensive research is needed to confirm or reject these hypotheses.

The “outgroups” that would be required to represent a time scale do not exist and it was thus not possible to estimate the separation times of the individual clones. It is therefore also not possible to establish which clone separated from which progenitor clone at which point in time. However, the results of the analyses indicate that the LEE was inserted by the individual clones at several different times.