6 Summary

Infections with avian pathogenic *E. coli* (APEC) cause colisepticemia, an acute and largely systemic disease resulting in significant economic losses in poultry industry worldwide. Although various virulence associated genes have been identified in APEC so far, their actual role in pathogenesis is still not fully understood and, furthermore, certain steps of the infection process have not been related to previously identified factors. Here the application of a Signature-tagged transposon mutagenesis (STM) approach is described to identify critical genes required for APEC infections in vivo. Due to the literature, this is the first time STM has been used for the study of *E. coli* infections of the respiratory tract leading to septicaemia of chicken.

To study pathogenesis in vivo, initially an infection model was established. The highly pathogenic strain IMT5155 (O2:H5) was elaborated, that had been shown to cause high mortality rates in 6-month-old chicken and typical symptoms of colisepticemia before. This strain belongs to the most common O-serotype (O2) associated with colisepticemia and represents a member of an important clonal lineage of APEC field isolates in Germany. For infection studies, intratracheal infection has been applied since it is more representative of the natural route of infection, is non-invasive and can be easily performed. An infective dose of 10^8 CFU was able to reproduce the disease in 85% of the animals and obtained optimal parameters for STM studies.

The original STM strategies were modified to use nonradioactive detection methods for its safety, ease of use and reduction of hazardous materials. 90 transposons with strong and specific signals when hybridized were pre-screened. The most significant reduction in background of hybridization was achieved by generating tag sequences with PCR as the target DNA for dot blots rather than using the entire plasmid containing the tagged transposon.

Twenty pools of about 1.800 IMT5155 (O2:H5) mutants were screened in the infection model and potentially attenuated mutants were subjected to a secondary screen and in vivo competition assays to confirm their attenuation. 1.7% (30/1800) of the total pool of mutants was confirmed to be attenuated for survival in internal organs. Four mutants have disrupted genes involved in different steps of LPS-biosynthesis and are required for the expression of a complete LPS molecule. This confirmed the importance of LPS including O-antigen in the pathogenesis of APEC. Another four mutants with insertions in genes involved in the synthesis of several distinct extracellular polysaccharide structures including group II capsule and colanic acid are attenuated in the ability to cause septicemia in a chicken model. Therefore, further evidence is provided for the importance of K1 capsule in APEC pathogenicity and for the first time, associated colanic acid with virulence and fitness of this pathogen. Iron acquisition systems have been associated with bacterial virulence especially for bacteria causing septicemia. Here, two novel genes encoding iron transporters in APEC that have not been previously characterized in APEC in in vivo studies, are identified. The importance of synthesis of vitamin precursors for the virulence of bacteria in vivo is also well established by identification of two mutants with interruptions in genes directly or indirectly involved in the synthesis of vitamins. In addition, several membrane and periplasmic proteins, metabolic enzymes, putative proteins with unknown function and *orf*s with no similarity to other data base entries were identified. This genome-wide analysis has identified both novel and previously known factors potentially involved in pathogenesis of APEC infection.

Future experiments are needed to functionally analyse the disrupted genes to elucidate the mechanisms by which they exert their effects as well as complementation and construction of defined-deletion mutations to confirm the observed attenuation. These studies will hopefully lead to a more comprehensive understanding of APEC virulence.