Summary

Molecular epidemiological investigations with avian pathogenic *Escherichia coli* (APEC) – field strains and establishment of a diagnostic multiplex polymerase chain reaction

Colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC) is an acute disease of poultry resulting in high morbidity and mortality and significant economic losses in poultry industry in many parts of the world. The differentation between infectious avian pathogenic *E. coli* and apathogenic *E.coli*, living as commensales in the intestinal tract of animals, is a major problem. Thus, only limited epidemiological data on APEC-infections are availaible. Furthermore, the role of distinctive APEC virulence factors have not yet been elucidated, although several in vivo- and in vitro-investigations were performed. Therefore we investigated 150 *E coli* strains isolated from visceral organs of poultry having died from colibacillosis during the last 10 years in germany. All *E. coli* isolates were tested for the presence of the serogroups O1, O2 and O78:K80, as these serogroups have been published to be mainly involved in avian colibacillosis. In addition, we investigated these strains for the presence of virulence-associated genes by polymerase chain reaction (PCR) and DNA-DNA-hybridization and examined their clonal relationship elaborating macrorestriction analysis.

Unexpectedly, only less than fifty percent of the investigated isolates represented the above mentioned serogroups O1 (6.0 %, 9 strains), O2 (28.7 %, 43 strains) and O78:80 (14.7 %, 22 strains). To get more insigths in the distribution and the occurrence of the virulence-associated genes described for colibacillosis we examined all 150 *E. coli* strains for the presence of the following genes: *fimC*, *papC*, *fyuA*, *irp2*, *iucD*, *iss*, *tsh*, *astA*, *hlyE* and *stx2f*. The genes *fimC* and *papC* coding for Type I- and P-fimbriae were present in 92.7 % (139 strains) and 23.3 % (35 strains) respectively. *FyuA* and *irp2* were regularly detected in combination in 71.3 % (107 strains) and *iucD* was found in 77.3 % (116 strains). 66.0 % (99 strains) of the investigated *E. coli* isolates harbored all three mentioned genes, which encode iron acquisition systems. In addition to the acquisition of trace elements essential for the growth of APEC in the host, the high prevalence (80.7 %, 121 strains) of *iss* (increased serum survival) conferring resistance to serum and phagocytosis, suggested to be also important during pathogenesis of colibacillosis. A further 72.0 % (108 strains) of the investigated *E. coli* strains gave positive results for the *tsh* gene that encodes the temperature sensitive hemagglutinin. *Iss* and *tsh* are regarded as specific genetic markers for avian pathogenic

E. coli strains. The product of *astA*, EAST-1 (enteroaggregative stable toxin) was found in 20.7 % (31 strains). The *hlyE* gene encoding hemolysinE could be detected only in 2.7 % (4 strains) of all investigated 150 *E. coli* isolates. In addition none of our field strains was positive for the pigeon-specific shiga toxin variant stx2f, pointing against any relevance of this particular gene during colibacillosis. On the basis of these results a multiplex polymerase chain reaction was established as a diagnostic tool for a specific, fast and cost-efficient identification and characterization of APEC field strains. Furthermore, macrorestriction analysis of the 150 APEC strains isolated from colibacillosis infections revealed a limited number of clonal lineages only.

These findings provide new insights into the presence and distribution of virulence-associated genes in avian pahogenic *E. coli* field strains. On the basis of this knowledge a specific diagnostic tool in form of a multiplex polymerase chain reaction has been established for the first time. In contrast to serotyping as a diagnostic tool, displaying a rather low sensitivity in the diagnosis of APEC, this multiplex PCR allows a specific identification. Furthermore, this multiplex PCR is a tool to estimate the virulence of each single avian pathogenic *E. coli* tested in the field. Thus highly virulent APEC strains identified in single outbreaks in a flock can be utilized as seeds for the production of farm specific vaccines. In addition to their role as avian pathogens, this typing gives more information about the strain´s ability to be a potential zoonotic pathogen, since human and avian pathogenic *E. coli* can easily compared with each other applying macrorestriction analysis established here. The established tools can also be applied to investigate infection chains, which would give valuable information about the pathways of infections in and in-between poultry flocks. Such analysis could be used for monitoring preventive measures against the spread APEC at an early stage of infection. Finally, the results obtained in this work give the basis for further epidemiological investigations and upcoming in vivo- (chicken) and in vitro- (cellculture) infection experiments, which will provide substantial information for a better understanding of the pathogenesis of colibacillosis in poultry.