Summary

Infections by *Salmonella* and avian pathogenic *Escherichia coli* (APEC) are responsible for major economic losses in poultry breeding. *Salmonella enterica* serovar Enteritidis (*S. Enteritidis*) and *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) in poultry meat and eggs are in addition major sources of human salmonellosis. APEC causes extra-intestinal infections in poultry with up to 20% mortality.

APEC initially infects the respiratory tract of poultry but can further disseminate through the body, infect internal organs and cause septicaemia. The prevention of infection by environmental control measures is not sufficient to eradicate the pathogens. Treatment with antibiotics should be reduced, since it causes an increase in multi-resistant pathogenic strains. A valuable alternative for antibiotic treatment is prevention of infection by vaccination.

Different kinds of vaccines were generated to protect poultry against *Salmonella* and APEC. Until today, no vaccine is available that induces full protection against challenge with homologous and heterologous pathogenic strains. A major drawback of the use of whole-cell vaccines is also that vaccinated animals and animals infected by a field strain cannot be distinguished by serological tests.

During this study, auxotrophic *S. Enteritidis* and *S. Typhimurium* mutants were obtained by deletion of the *aroA* gene or the *guaB* gene. Both mutations were previously described as attenuating. Virulence tests in mice demonstrated that after oral immunisation with a high dose of the *guaB* mutant, symptoms of disease were induced and that mice inoculated with the *aroA* mutant were asymptomatic. Protection induced after immunisation with the *guaB* mutant was at least as strong as protection induced after immunisation with the *aroA* mutant.

Flagellin, the major structural protein of flagella, is a strong antigen that is used for serotyping of *Salmonella*. The genes encoding flagellin, *fliC* and *fljB* (two flagellins are present in *S. Typhimurium*), were deleted in the attenuated strains, to generate non-motile derivatives. Thereby, a bacterial (non-motility) and a serological (absence of antibodies elicited against flagellin) marker was introduced in the vaccine strains. The influence of the inactivation of the flagellin genes on the virulence and efficacy of the vaccine strains was determined after oral immunisation of mice. Disease symptoms induced in mice, after inoculation with the *S. Enteritidis ΔguaB* mutant, were reduced after inactivation of flagellin genes in the auxotrophic *Salmonella* mutants and at the same time the efficacy of protection
increased. The S. Enteritidis ΔguaBΔfliC mutant is safe in one-day-old chicks, in contrast to the ΔguaB mutant. The inactivation of flagellin genes as a serological marker in live vaccines should be further evaluated.

Prior to the construction of live attenuated APEC vaccines, a study was performed to identify factors that contribute to the virulence of APEC and that can be used as candidate protective antigens in vaccine development. Different studies compared E. coli isolates originating from chickens affected by colibacillosis with isolates derived from healthy chickens for the presence of putative virulence factors. Factors contributing to the virulence of extra-intestinal E. coli include iron scavenging mechanisms, serum resistance, adhesins and temperature sensitive hemagglutinin. The presence of a colicin V producing high molecular weight plasmid (ColV) is associated with virulence, due to the presence of virulence-associated genes on the plasmid, such as tsh (encoding temperature sensitive hemagglutinin), iuc/iut (encoding the aerobactin siderophore system), iro (salmochelin), iss and traT (determining serum resistance). Our data, obtained from 80 Belgian strains, isolated from colibacillosis-affected chickens and chickens from control flocks, confirm these results. Also the presence of three newly identified ABC transporters (eitABCD, etsABC and sitABCD) was investigated. Isolates that belong to the major serogroup O78 all harbour a ColV plasmid with associated genes tsh, iutA/iucA, iroC, sitA, etsB, iss and traT. None of the O78 isolates were positive for vat (vacuolating autotransporter toxin), ibeA (invasion of brain microvascular endothelial cells) or eitA. Serogroup O2 is more diverse and not all isolates contain a ColV plasmid with associated genes. All O2 isolates are positive for ibeA, sitA and iss. Some possess etsB, eitA and vat.

For the construction of live APEC vaccines two strategies were followed. One strategy was to attenuate APEC strains by deleting the aroA or guaB gene, as was previously done for Salmonella. This was performed in strains APEC19E, of the major serotype O78, and APEC1, a serotype O45 strain known to be virulent in a chicken challenge model. Both strains harbour a ColV plasmid with associated virulence traits. The O-antigens can contribute to the induction of protection against homologous challenge, as was previously demonstrated for the O78-antigen.

Mice were inoculated orally, nasally and intraperitoneally with the wild type APEC strains and the virulence and immunogenicity of the strains was determined. All mice survived without showing disease symptoms, what demonstrates that adult mice cannot be used for
challenge experiments with these strains. APEC1 was virulent in neonatal mice, after intranasal infection and can therefore be used in a challenge model. Newborn mice of mothers immunised nasally with APEC1 or the derived mutants, were partially protected against challenge with APEC1. This shows that protection can be accomplished by maternal antibodies.

Some mice immunised nasally or intraperitoneally with wild type APEC showed an IgG response against Tsh, but not against IutA and PapGII. No anti-Tsh response was detected in sera from mice immunised with the auxotrophic strains. Since also the auxotrophic mutants induce protection against homologous challenge, Tsh is probably not the main protective antigen in mice. Anti-LPS IgG was detected in sera of all immunised groups. The importance of anti-LPS antibodies for the protection against homologous APEC strains was previously shown.

Dual candidate live vaccines were constructed by the introduction of the ColV plasmid of an APEC strain in an attenuated S. Enteritidis carrier strain. Sera of mice immunised intranasally with the dual vaccine strains were tested for the presence of IgG against outer membrane proteins of APEC. No IgG response was detected against Tsh. An anti-TraT IgG response was observed after boost in serum of one mouse. Neonatal mice, delivered by mothers immunised with the S. Enteritidis vaccine strain harbouring ColV, and with the parental S. Enteritidis vaccine strain as a control, were challenged with APEC1. No significant difference was observed between both groups and the non-infected controls. This shows that immunisation of mothers with the S. Enteritidis vaccine strain harbouring ColV does not protect the delivered neonatal mice against challenge with pathogenic APEC.