Construction of a Recombinant Escherichia Coli Strain for the Development of a Stx2e Subunit Vaccine against Edema Disease

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Introduction

Edema disease of pigs occurs worldwide and is usually a peracute to acute systemic disease. It appears mainly in piglets during the first two weeks after weaning. Edema disease E. coli (EDEC) strains produce two virulence factors, which are crucial for pathogenesis. The formation of F18 fimbriae is necessary to allow the massive proliferation of EDEC in the small intestine. Subsequent secretion of shiga toxin 2e (Stx2e) in the intestines, which is then resorbed and spread through the organism is the cause for toxic endothelial damage. In the endothelial cells Stx2e causes inhibition of protein biosynthesis, which leads to cell death. This results in lesions of small arteries and arterioles followed by edema and damage to the central nervous system. Active immunisation is seen as a strategy for the prevention of edema disease. Therefore, the aim of this work was to develop an E. coli strain, which expresses an immunogenic Stx2e antigen with highly reduced cytotoxicity. Furthermore, this strain should be free of any antibiotic resistance marker for selection and plasmid stabilization.

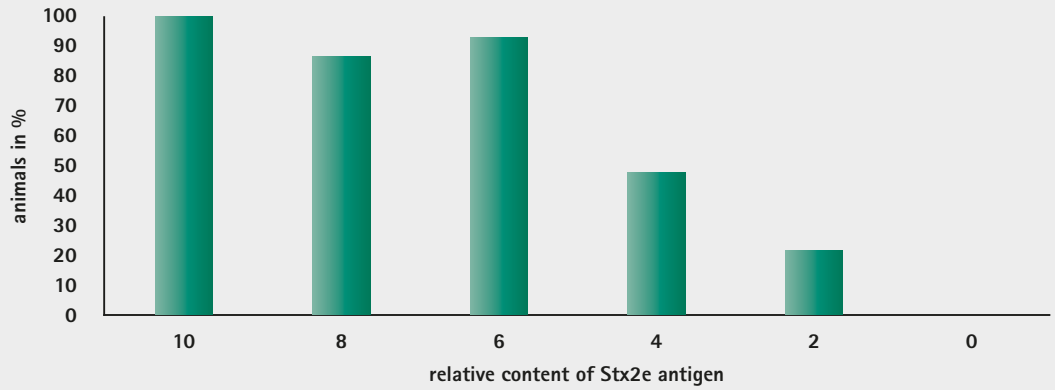
Materials and Methods

An E.coli K12 strain was chosen as starting strain for the genetic construction. Deletion of an essential gene was performed to obtain a selectable marker. After PCR amplification, this marker gene and the genes coding for the subunits A and B of Stx2e were ligated in an expression plasmid. The stx2eA gene was modified by site directed mutagenesis to produce a Stx2e antigen with reduced cytotoxicity. Expression of shiga toxin 2e antigen and cytotoxicity were tested in an ELISA and a vero cell cytotoxicity assay. Toxicity of the genetically modified Stx2e antigen to piglets was tested in animals on the fourth day of life. The ability of a vaccine on the basis of the constructed strain and with aluminum hydroxide as adjuvant to induce Stx2e neutralizing antibodies was investigated by a single shot immunization of piglets on the fourth day of life with vaccine preparations containing different amounts of Stx2e antigen. The presence of neutralizing antibodies was investigated by a serum neutralization assay on vero cells.

Results

An E. coli K12 strain with a chromosomal deletion of an essential gene was successfully generated. By replacing the resistance marker of an expression plasmid with this essential gene a plasmid selection and stabilization system was established, which is not dependent on antibiotics. The genes coding for the two subunits of Stx2e were ligated in this plasmid and the sequence of the stx2eA gene was genetically modified. The stable plasmid maintenance of the resulting plasmid was demonstrated by sub-culturing the strain over 100 generations. The modified Stx2e antigen is expressed at high level in the culture supernatant and it was shown that its cytotoxicity is reduced by more than 4 orders of magnitude compared to the unmodified toxin. It was shown that the application of high amounts of the modified Stx2e antigen to piglets on the 4th day of life did not result in inducing typical clinical signs of edema disease. To produce a vaccine intact plasmid present in the culture supernatant after fermentation is chemically degraded. Immunization of piglets with a vaccine containing the modified Stx2e antigen on the fourth day of life results in the induction of neutralizing antibodies in a dose dependent manner (Figure 1).

Figure 1 Percentage of animals with Stx2e neutralizing antibodies 21 days after immunization with different amounts of Stx2e antigen.



Conclusions and Discussion

The construction of a recombinant E. coli strain bearing a plasmid, which codes for a modified Stx2e antigen is presented. The plasmid is stably maintained without the use of antibiotics and the cytotoxicity of the Stx2e antigen is highly reduced. A single immunization of piglets on the fourth day of life induces the generation of Stx2e neutralizing protective antibodies at the time of weaning. This strain is the basis for the development of a vaccine against edema disease, which is considered to be safe for the animals, the user and the environment. ▪