

## 7. Summary

### **Effects of a probiotic strain of *Enterococcus faecium* on natural infection rate of *Chlamydiae* in swine**

*Chlamydiae* are obligate intracellular pathogens which cause infections associated with a broad range of diseases in both livestock and humans. Epidemiologically persistently clinically inapparent infected animals are of particular importance. As a result of persistent infection these animals become a reservoir of pathogens for other animals, shedding these potential zoonotic pathogens. It is therefore of utmost importance to definitively diagnose infection on a species-specific level and to decrease the chlamydial load of these animals.

The aim of this study was to examine the gut as a habitat of *Chlamydiae* utilizing conventional as well as modern molecular tools for determination of possible latent chlamydial infection. Cell cultures were used for cultivation of *Chlamydiae* from faeces or mucosal samples from swine. Species-specific polymerase chain reactions (PCR) were applied on samples from ileum, colon and faeces. To confirm active infection, immunohistochemistry (IHC) and fluorescence-*in-situ*-hybridisation (FISH) procedures were carried out with samples from the small and large intestines. In the course of the FISH examinations, a hierarchic oligonucleotide probe set was applied for the first time on tissue to demonstrate intestinal chlamydial infection.

Reason of systematic examination was to perform a study comparing the efficacy of feeding with a probiotic strain of *Enterococcus faecium* NCIMB 10415 on the rate of natural infection of *Chlamydiae* in gut of swine. To determine this, we performed a study comparing two groups of animals, one that was fed with *Enterococcus faecium* (probiotic group) and one that did not receive probiotics (control group). Initially the carrier status of sows (n = 22) was determined in both feeding groups, then the piglets of *Chlamydia*-positive sows were examined systematically for carry-over infections and potential differences between these two groups. Therefore 4 piglets from each of five *Chlamydia*-positive sows (n=20 piglets) from either the control or probiotic group were examined for the frequency of *Chlamydia* at ages of 14, 21, 35 and 56 days *post partum*.

In the examined population of conventional sows a high rate of latent *Chlamydia suis* infection could be determined. A clear difference in infection rate in the comparison of both groups could be detected. 85% (17/20) of the piglets from the control group were found to be *Chlamydiae*-positive by PCR, whereas *Chlamydiae* were found in only 60% (12/20) of piglets

from the probiotic group. These results were confirmed by a higher infection rate of ileal and colon tissues by FISH and immunohistology. In addition to the reduced frequency of *Chlamydiae*-positive piglets in the probiotic group, the time point of infection was also delayed.

Cell cultivation was not found to be a reliable diagnostic method for determining latent chlamydial infection of pig gut. The elaborated species-specific nested-PCR with detection limit of <1 IFU/ml was the most sensitive technique. The comparison of techniques for *in-situ* detection of *Chlamydiae* showed that IHC and FISH could be considered equivalent with regard to identification frequency. The advantage of FISH in comparison to IHC was the possibility to acquire a species-specific diagnosis using the developed hierarchic probe set.