8 Summary

Prevalence of thermophilic *Campylobacter* spp. in broiler flocks with follow-up of flock specific clones from farm through slaughter to retail products using pulsed field gel electrophoresis (PFGE)

In Germany campylobacteriosis is the second most frequent cause of gastro-intestinal infections. In 2003, a total of 47,876 cases of campylobacteriosis in humans were recorded. Campylobacteriosis in man is mainly a food-borne infection, in which foods of animal origin, and in particular raw or undercooked poultry meat play an important role.

Against this backround German broiler flocks were screened to assess their campylobacter carriage. In addition, the spread of *Campylobacter* spp. during the slaughtering process was surveyed. Possible hygienic barriers in the processing line were also investigated.

The main focus of this study was the detection of same genotypes in broiler flocks on farm level as well as in the slaughtering process up to the end-products. Therefore broiler flocks were sampled on farm level and at various stages throughout processing. Selected campylobacter isolates were genotyped by pulsed-field gel electrophoresis (PFGE).

In the period December 2001 to August 2002, 510 samples from fresh droppings of 51 flocks located in 3 farms of different geographical regions were analyzed for the presence of thermophilic campylobacters. In a second step 1101 samples were taken from these flocks on different stages of processing and also analyzed for *Campylobacter* spp. Sampling sites included: transport crates before and after cleaning/disinfection, post-scalded and post-chilled carcasses, evisceration (ceca, giblets) and end-products. Additionally, some selected water samples (washing water of the transport crates, stunning water, scald water, water of the liver-gut-tub) were analyzed for *Campylobacter* spp. before and after slaughtering of the flocks.

Isolation of *Campylobacter* spp. was performed in general accordance with the guideline ISO 10272. Altogether 237 isolates were subtyped by PFGE, using the restriction enzyme *Sma*I. 103 selected strains were additionally analyzed using the restriction enzyme *Kpn*I.

Campylobacter carriage of flocks showed seasonal variation, with the highest contamination rate (100%) during the period June to August.

Campylobacter spp. was isolated from 23 (45.1%) out of the 51 broiler flocks examined. Among the campylobacter positive samples, *C. jejuni* was diagnosed in 76.1% of the cases and *C. coli* in 23.9% of the cases. 9.6% of the isolates were nalidixic acid resistent. Quinolone resistence in *Campylobacter* spp. and consequent implications for the treatment of human diarrhoea are described in the literature.

In general, all 10 samples of fresh droppings taken in the front, middle and back area of the broiler house were positive once colonisation had been proven. This leads to the conclusion, that once campylobacter had entered a flock, all broilers became colonized very fast, because of a rapid spread within the flock. Although the hygienic measures (e.g. use of separate clothes and boots for the broiler houses) in the broiler farms differed, no significant difference concerning contamination of the broiler flocks with *Campylobacter* spp. was observed. In the period June to August every sampled flock was contaminated with *Campylobacter* spp. The results indicate that there were not enough hygienic barrieres to prevent contamination of the flocks. No evidence was found for a horizontal transmission from one broiler flock to the next via a persistent house-contamination. It seems to be obvious that the major route for campylobacter contamination of the broiler flocks was a horizontal transmission from the environment. The transmission of the organism between subsequent flocks has probably been prevented by efficient cleaning and disinfection.

In most flocks, specific campylobacter strains could be detected. In each positive flock one to three different genotypes were found. The result confirmed that individual broiler flocks are colonized by a limited number of clones of *C. jejuni* or *C. coli*. One or two clones were dominating others on farm level as well as in the slaughtering process. This result indicated that some campylo-bacter clones are more robust and survive poultry processing better than others. The fact, that in different flocks isolates of clonal origin could be detected during the same rearing period, suggests a transmission between the broiler flocks or an intermittent common external campylobacter source. In some cases isolates of clonal origin could be detected in various farms during different rearing periods. The reason for that was probably a cross-contamination between the farms. This result suggests that some clones of

Campylobacter spp. remain genetically stable over a longer time period and geographical distance.

At the abattoir, the massive spreading of *Campylobacter* spp. when slaughtering a positive flock was proven. Sampling during processing confirmed that the entrance of a positive flock resulted in contamination of the abattoir environment. *Campylobacter* spp. were isolated from all sampling stages along the processing line, with a percentage of 91.1% to 100% of isolates at different stages of slaughtering. No hygienic barriers against the spread of campylobacter during the slaughtering process could be detected. Neither scalding temperature (50°C to 53°C) nor drying of the carcass-surface could prevent the spread of *Campylobacter* spp. during slaughtering of positive flocks. All samples of livers, guts and hearts and 96.7% of the poultry carcasses examined after slaughtering positive flocks, were contaminated with *Campylobacter* spp. The survival of *Campylobacter* spp. at cooling temperatures suggested a possibility of human campylobacteriosis caused by these products.

Washing water of transport crates, stunning water, scald water and water of the liver-gut-tub were partly contaminated with *Campylobacter* spp. before slaughtering and generally after slaughtering positive flocks. The cleaning and disinfection of transport crates was insufficient. After the cleaning and disinfection, 91.1% of transport crates were still contaminated with *Campylobacter* spp.

Most isolates at the abattoir belonged to the same genotype as the farm isolates from the flocks slaughtered. It was possible to detect flock-specific clones in the farms as well as in the abattoir by using the PFGE for genotyping. This result indicates that positive flocks are responsible for the contamination of different stages in the abattoir. By using the PFGE it was also possible to detect flock-specific clones on contaminated giblets and end-products. In some cases the same genotypes isolated from faeces contaminated the end-products.

It appeared that some campylobacter clones can carry over onto the carcasses of subsequent flocks. This observation was confirmed by using the PFGE. Therefore it can be suggested that cross-contamination between different flocks can occur during processing.

In this study PFGE appeared to be useful for epidemiological analysis of strains of *C. jejuni* and *C. coli*.

The results of this study showed that, especially in the summer months, up to 100% of the sampled broiler flocks were colonized with *Campylobacter* spp. at the time of slaughter. Therefore, the cross-contamination of broiler carcasses presented a potential hygiene problem when campylobacter-free flocks follow colonized flocks through the processing plant. The significance of campylobacter contaminated poultry-products as relating to the potential for infection of consumers, through the consumption and handling of poultry, is recently established as a significant risk factor in the transmission of campylobacters to humans.

Therefore, intervention-procedures at farm level have to be studied further. The effectiveness of strict hygienic practices in farms as well as in abattoirs has to be evaluated. Also, adequate consumer information on proper handling of poultry meat is needed.