Campylobacter in pigs: an epidemiological study

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Summary: To improve our knowledge of the epidemiology of Campylobacter in pigs and to know the prevalence of these bacteria in pigs around the area of Leipzig, we monitored 60 fattening pigs and 30 piglets. Faeces samples were collected at different times and at the slaughterhouse. Till now 376 isolates could be detected, and all were proved as *C. coli*. The prevalence of campylobacter in fattening pigs is between 70 and 93%. All the piglets are negative at the day of birth, but the rate of Campylobacter-positive piglets increased up to 90% after 3 weeks. For epidemiological studies we used two high-resolution genomic fingerprinting methods (AFLP and RFLP) to characterize the isolates at the strain level. We could distinguish 45 AFLP pattern types and 15 RFLP pattern types. Both methods are proved to be useful tools for epidemiological investigations, but the discriminatory power of AFLP is higher.

Keywords: Prevalence, Genetic diversity, AFLP, RFLP

Introduction: In recent years the infection with Campylobacter in developed countries has increased. In some countries (GB, NL, DK, USA) Campylobacter advanced to the leading cause of bacterial gastro-enteritis and has displaced salmonellas from their leading position. Especially the thermotolerant species of the genus Campylobacter, *C. jejuni* (90%) and *C. coli* (5-10%) are the most important cause of campylobacteriosis in humans. Pigs are probably one of the natural reservoirs of Campylobacter, and they excrete them with their faeces. The predominant species that colonize swine is *C. coli*. In this study we wanted to get some knowledge about the epidemiology of Campylobacter spp. in pigs.

Materials and methods: The excretion pattern of Campylobacter spp. of 60 fattening pigs was documented over a fattening period. Every pig was marked with a numbered blue ear tag for identification among the herd. Porcine faeces were collected rectally with sterile plastic bags at day 1 of the beginning of the fattening period and after 4, 8, and 12 weeks. At the slaughterhouse a piece of the colon was cut out sterilly to get faeces. To detect possible sources of entry, environmental
samples (rodents, feed, water, etc.) were investigated. To see how piglets get colonized rectal swabs were taken from sows and from their piglets (30 numbered and marked piglets) at the day of birth and after 1, 2, and 3 weeks, after removal from the sow to the nursery and in the fattening department. The samples were investigated bacteriologically. Strains who showed characteristic features of campylobacter were frozen at –84 °C and the DNA was extracted (DNA Purification kit, Amersham). Two genotyping methods were used and compared for the following identification: a flaA PCR-RFLP protocol with some modifications (Nacihanikin et al., 1996) and a modified AFLP protocol (Duim et al., 1999). Gels were stained with ethidium bromide and documented under UV illumination with alpha ease software (alpha innotech). The resulting AFLP and RFLP patterns were compared using Phoretix 1.0 advanced and database software (nonlinear dynamics).

**Results:** Till now 376 isolates could be found in the pigs and all were identified as *C. coli*. The prevalence in fattening pigs is between 70 and 93% during the whole period and at the slaughterhouse. All the newborn piglets are negative, but the rate of Campylobacter-positive piglets increased up to 90% after 3 weeks. All dams of the piglets also excreted *C. coli*. Some pigs excrete *C. coli* at all sampling times, others excrete them intermittently. In one rat we could find *C. coli*, all other environmental probes were found to be Campylobacter-negative. AFLP fingerprints showed about 25 to 30 fragments in the range of 50 to 500 bp, while RFLP fingerprints had 4 to 8 fragments in the range of 100 to 1000 bp. The genetic homologies between the strains were analyzed by using Phoretix 1.0 advanced and database software for cluster analysis. From our knowledge we designated all strains with a similarity between 95 and 100% homology as being identically and with a similarity from 90 to 95% as being genetically highly related. This limit gave rise for 45 different AFLP patterns and 15 RFLP patterns. In the dendogram clusters of pigs, belonging to one compartment of the stable and to the time of sampling, can be differentiated. Some pigs excrete the same strain at all sampling times and others excrete different ones at different sampling times.

**Discussion:** In this study we investigated the excretion pattern of Campylobacter spp. in pigs. The prevalence in fattening pigs is very high: between 70 and 93% during the whole fattening period and at the slaughterhouse. Piglets were negative at the day of birth, but the rate of Campylobacter positive increased up to 90% after the removal from the sow and later in the fattening department. Some pigs excrete *C. coli* all the time, others excrete them intermittently. These high rates of Campylobacter-positive pigs are comparable with results of other groups (Weijtens et al., 1993; Young et al., 2000). It can be estimated that the source of entry is from dam to offspring. All strains were identified phenotypically and genotypically as *C. coli*. The genotyping showed high similarity between the
strains. Of the 376 C. coli strains, 40 different AFLP and 15 different RFLP patterns were obtained. The diversity of the types observed in this investigation, not only within the population as a whole, but even within individual faecal samples, could have different reasons. It is very probable that the fattening pigs became infected with different types before arrival at the fattening compartment (the mothers also excrete various types) and later they get infected with different types from other sources (other pigs, farmer). But it also could be explained by a number of characteristics of the campylobacter genome. The ability to change and rearrange the genomic structure fast, results in the change of the original genome. The AFLP and RFLP techniques are a useful and practical tool with a good discriminatory power for epidemiological studies. The advantage of AFLP over RFLP is that bands from all over the genome are derived, while the RFLP technique is focusing on one gene. To understand what role the strains, found in pigs, play for possible sources for infection of humans we will compare our strains with Campylobacter strains, especially C. coli, from humans and from other sources.