The bacterium *Campylobacter jejuni* naturally colonizes the gastrointestinal tract of many birds and animals, resulting in a harmless commensal relationship, but this microbe can also promote pathogenesis in humans, resulting in a productive gastroenteritis leading to a mild bloody diarrhoeal syndrome. Consumption or handling of poultry meats is prevalent source of *C. jejuni* for infection in human. However, the majority of *C. jejuni* infections are considered to be sporadic, with the source of infection remaining unidentified. It is unclear whether certain types of *C. jejuni* strains are specific for particular hosts or whether they are associated with specific disease manifestations in humans. Genotyping performed by using two methods provided insights into the genomic relatedness of isolates from different broiler flocks from extensional farms with conventional rearing technology. 319 samples from 19 different flocks were clearly discrimined by PCR/RFLP *fly*A-typing (*fla*-RFLP) as well as by subtyping performed using pulsed-field gel electrophoresis (PFGE) and the enzyme used for cleavage was *Sma*I. One flock was colonized only by *C. coli* clone with identical PFGE profile. In other 18 flocks 17 *fla*-RFLP and 22 PFGE *C. jejuni* subtypes were found. In seven cases each individual flock was colonized by clones with identical profiles both after *fla*-RFLP typing and PFGE analysis. In three flocks *C. jejuni* strains with identical PCR/RFLP profile and only similar PFGE patterns were found. On the other hand, four flocks were colonized by strains with identical PFGE profiles, but different PCR types. Nevertheless all these *fla*-RFLP types in one flock can be explained by possible transition of one subtype after mutation of the recognition site for only one restriction enzyme. Based on identity or significant PFGE similarity and in most cases *fla*-type identity, we assume that *Campylobacter* isolates from each of the 14 flocks are homogenous and belong to the same clone. Four flocks may be colonized by 2 - 3 different *Campylobacter* clones with quite different PFGE profiles and *fla*-RFLP types which could not be explained by point mutation in restriction site. The bacterial population in poultry appeared to be mostly homogenous – even though in three flocks isolates contained mixture of two *C. jejuni* clones with similar PFGE profile and identical *fla*-type and vice versa. To determine the significance of poultry as infectious source of *C. jejuni*, the PCR/RFLP and PFGE patterns of broiler chickens are compared with those of isolates from patients with diarrhea, at present.

**Keywords:** *Campylobacter jejuni*; PCR; PFGE; subtyping

**Introduction**

Infections caused by thermophilic *Campylobacter* sp. in man are quite common. These microorganisms together with *Salmonella* sp. are the most common causes of diseases originating from food in the Czech Republic. Though the pathogen seems to be rather sensitive and appears to have substantial requirements for survival and replication in food, increasing numbers of human ailments have been observed in the Czech Republic. The reasons for constantly increasing occurrence
are not fully understood, though raw poultry meat and inadequately heat-treated poultry products are considered to be a potential source of infection (Gerdemann, 1996). A number of poultry farms and chicken being slaughtered appear to be contaminated by *Campylobacter* sp. and during treatment in the slaughterhouse surface contamination can occur. Unhygienic handling of raw poultry meat results in contamination of other foodstuffs. The risk of ailments arises also from insufficient treatment of poultry meat and semi-finished poultry products (Berrang et al., 2004). The frequency of *Campylobacter* sp. incidence in poultry varies significantly at particular farms and depends on a number of factors, primarily on zoo-hygienic circumstances (Meldrum et al., 2004; Skov et al., 2004).

In spite of the frequency of *Campylobacter jejuni* findings in poultry, the degree of resistance of individual *C. jejuni* strains in the environment remains unclear. Moreover, it is not quite obvious if only particular types of *Campylobacter* sp. occur in poultry, or whether strains isolated from humans are identical with those isolated from poultry. The frequent collection of samples from patients and the improved diagnostics of pathogens may also be among the factors contributing to the increased number of reported cases. Recently, techniques of molecular typing, primarily pulsed-field gel electrophoresis (PFGE), PCR-restriction fragment length polymorphisms of the flagellin gene (fla-RFLP), amplified fragment length polymorphism (AFLP), and ribotyping have been used to identify *C. jejuni* strains (de Boer et al., 2000; Nielsen et al., 2000; Kärenlampi et al., 2003; Guvérémon et al., 2004). Genome instability could result in genotype variability and thus in problems in the interpretation of epidemiological studies, particularly when only one technique has been used.

In the course of the last few years we focused on the assessment of *Campylobacter* sp. occurrence in slaughtered poultry and in the slaughterhouse environment. Some farms have been sampled repeatedly, and in these farms typing of isolated strains and comparison of subtypes was performed. The aim of this study was to discover the circulation of *C. jejuni* strains in selected broiler farms.

**Materials and methods**

343 samples from 19 broiler flocks were obtained from 13 extensional farms with conventional rearing technology. At nine farms the sampling took place only once, at two farm samples were taken twice in the course of one month, and at the two farm samples were taken three times during five months. The same day when the chickens were slaughtered, the samples of the digestive tract were taken. In the laboratory the caecum was cut aseptically and 1 g of caecal content was cultivated according to the ČSN ISO 10272 standard.

For PCR/RFLP analysis DNA from bacterial cultures was isolated by standard phenol-chloroform extraction (Sambrook et al., 1989). The specific identification and differentiation of thermophilic *Campylobacter* sp. were performed by using polymerase chain reaction in combination with the analysis of restriction fragment-length polymorphism (PCR/RFLP). The polymorphic region of the 23S rRNA gene was amplified to yield the PCR product 491 bp long. The primers used are specific for the group of thermophilic *Campylobacter* sp. while the amplification of other *Campylobacter* sp. or other bacteria does not occur. Subsequent cleavage with the restriction endonuclease *Alu*I gave unique combinations of fragments for *C. jejuni* and *C. lari*, respectively. Digestion with second enzyme, Tsp509I, was used for differentiation of *C. coli* and *C. upsaliensis* (Fermér and Engvall, 1999). 24 samples were identified as *C. coli* and 319 as *C. jejuni*.

For fla-RFLP sub-typing of *C. jejuni* strains, variable part of the flagellin A gene was amplified (Nishimura et al., 1996). Digestion with three restriction endonucleases gives several profiles of fragments: *Afa*I – six, *Mbo*I – seven, and *Hae*III – five types. By mutual combination of these types, 31 subtypes of *C. jejuni* have been found so far (Steinhauserova et al., 2002).

Further sub-typing of *C. jejuni* and *C. coli* strains was performed by PFGE analysis of the whole chromosome. Chromosome DNA was isolated in 1% agarose plugs incubated in ESP lysate buffer (0.5 mol 1⁻¹ EDTA, 1% laureyl sarcosine, 1mg ml⁻¹ proteinase K) at 50°C for 24 hours. Following a double rinse in TE buffer, plugs were used for digestion with restriction enzyme *Smal* in 100µl buffer (4U RE at 30°C). The DNA fragments were separated on CHEF-DR III system (BioRad) in 1% agarose in three steps at 200 V, angle 120° with pulse time of 5 -10 s for 4 h, 10 - 40 s for 14 hours and 50 - 60 s for 4 h at 9°C. PFGE results were evaluated using TotalLab software (Phoretix).
Results and discussion

From 19 broiler flocks 343 *Campylobacter* sp. strains were obtained. By PCR/RFLP 319 *C. jejuni* and 24 *C. coli* strains were identified. They were classified as particular subtypes by PFGE after digestion with *Sma*I. Individual poultry strains of *C. jejuni* were further analysed by fla-RFLP method. These methods are among the most discriminative ones (de Boer et al., 2000; Nilsen et al., 2000). The patterns of *Sma*I digestion are more stable than polymorphisms in the *flaA* gene. In flagellin locus the intragenetic and intergenetic recombination is rather common and may lead to variability in genotypes (Harrington et al., 1997). Individual subtypes obtained by fla-RFLP were marked with Arabic numerals (Steinhauserova et al., 2002) and for better orientation individual PFGE profiles were marked with letters. Since in the individual flocks samples from several animals were collected at one sampling, the homogeneity of the bacterial population could be assessed. One flock was colonized only by *C. coli* clone with identical PFGE profile (Fig. 1a). In other 18 flocks 17 fla-RFLP and 22 PFGE *C. jejuni* subtypes were found. From each individual flock at particular farms, 8–20 samples of *C. jejuni* were analysed. One-shot sampling at seven farms showed that each individual flock has been colonized with clones demonstrating identical profiles both after fla-RFLP typing and PFGE analysis. That means one bacterial clone had infected the whole flock and remained unchanged during transfer among individual hosts until the slaughter. However, the degree of stability of these clones cannot be identified, since the time of infection prior to sampling and the rate of infection spreading remain unknown.

![PFGE profiles of individual broiler flocks](image)

Figure 1 PFGE profiles of individual broiler flocks. a) flock 1: lanes 1-9 *C. coli*; b) flock 2:lanes 1, 3-8, 10 *C. jejuni* type F, lanes 2, 9 type F₁; c) flock 3: lane 1 *C. jejuni* type D, lanes 2, 12 *C. coli*, lane 3, 9 *C. jejuni* type C (C₁), lanes 4,5,7 *C. jejuni* type A (A₁), line 6 *C. jejuni* type K, line 8 *C. jejuni* type H, lanes 10, 11 *C. jejuni* type J; M – Lambda Ladder PFG Marker (48,5 – 1018,5 kb)

In three flocks *C. jejuni* strains with identical PCR/RFLP profile and only similar PFGE patterns were found (Fig. 1b). PFGE genotypes were considered closely related if they differed by one to three bands and were considered indistinguishable if they had all bands in common (Tenover et al., 1995). PFGE analysis suggests that during the colonization, mutation at the restriction site for *Sma*I (used for PFGE) occurred. On the other hand, four flocks were colonized by strains with identical PFGE profiles, but different PCR types. Nevertheless all these fla-RFLP types in individual flocks can be explained by possible transition of one subtype after mutation of the recognition site for only one restriction enzyme used for RFLP analysis. Based on identity or significant PFGE similarity and in most cases fla-type identity, we assume that *Campylobacter* isolates from each of this flocks are
mostly homogenous and belong to the same clone. Ayling et al. (1996) suggest that majority of farms with one prevailing profile could arise from a single source of infection.

Four flocks may be colonized by 2, 3 or more Campylobacter sp. clones with quite different PFGE profiles and fla-RFLP types which could not be explained by point mutation in one restriction site. In three of this flocks C. coli strains were found, too. (Fig. 1c). Guévremont et al. (2004) have found several genetic profiles at a single farm, and even more than one genetic profile of C. coli in 50% isolates from one individual pig. On the contrary to Jacobs-Reitsma (1997) and Cardinale et al. (2006) we did not found the presence of one predominant strain at a particular farm.

The PCR/RFLP and PFGE profiles of two or three samplings taken from individual flocks in four farms in the course from one to five months showed significant diversity among strains which suggests different sources of infection. Poultry flocks appear to become infected mainly by horizontal pathways via the farm environment in spite of the use of hygiene barriers (Van de Giessen et al., 1998).

Our results imply the question of primary source of infection in the whole flock. Either the flock has already been the carrier of single bacterial clone, or more clones are present and during the colonization the type with higher vitality is cloned. Some strains of Campylobacter sp. are able to become dominant, while preventing colonization by other strains (Korolik et al., 1998). Hänninen et al. (1999) suppose that intestinal colonization can bestow an advantage to those genetic recombinants which become visible in the PFGE patterns. Examinations of samples with only one method is insufficient mainly for epidemiology studies, since seemingly different clones identified with one method could originate from a single clone, which could be proved with the other method.

To determine the significance of poultry as infectious source of C. jejuni, the PCR/RFLP and PFGE patterns of broilers are compared with those of isolates from patients with diarrhea, at present.

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References


