Prevalence of *Campylobacter* subtypes in pheasants (*Phasianus colchicus* spp. *torquatus*) in the Czech Republic

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ABSTRACT: At the turn of 2005 and 2006, cecal samples from 302 pheasants, from two flocks from a farm with intensive production, and from 200 wild birds were collected. The prevalence of *Campylobacter* spp. in the intestinal contents of pheasants from the farm was 70.20%. 50.47% of isolated strains were identified as *C. coli* and 41.40% as *C. jejuni*. In samples from wild pheasants, positive cultivation of *Campylobacter* spp. was proven in 27.5% of cases. A total of 15 PFGE subtypes were noted among the *Campylobacter* spp. isolates. *C. jejuni* strains could be divided into 16 *fla*-RFLP subtypes. No PFGE profile specific for *C. jejuni* and *C. coli*, respectively, was found. When comparing wild birds with farmed pheasants, there were eight possible combinations of PCR/RFLP and PFGE subtypes in wild birds and 40 combinations in farmed pheasants. No preferred combination of both subtypes was detected and all combinations were randomly ordered. To determine the significance of pheasants as a source of *C. jejuni* infection in humans, the RFLP and PFGE patterns of pheasant isolates are currently being compared with those of isolates from diarrheic patients.

Keywords: *Campylobacter jejuni*; *Campylobacter coli*; PCR/RFLP; PFGE

Campylobacter jejuni has been recognized as a cause of major health problems and it is connected with increasing incidence of bacterial enteritis. Undercooked poultry meat, infected water and unpasteurized milk have been identified as risk factors of the infection (Fitzgerald et al., 2001; Devane et al., 2005). *Campylobacter* spp. is frequently found in feces of healthy animals, especially birds and is widely distributed in the environment (Engvall et al., 2002; Workman et al., 2005). Wild birds are often considered to be a potential reservoir for *C. jejuni* in nature and possible source of human infections (Levesque et al., 2000; Waldenstrom et al., 2002). So far, however, genotyping methods were rarely used to examine whether bacterial isolates found in wild birds are comparable with those isolated from humans with clinical disease. Broman et al. (2002) found only a few strains from black-headed gull that had genotypic profiles identical to those from human strains. Broman et al. (2004) subtyped *C. jejuni* strains from migrant birds by pulsed-field gel electrophoresis. Isolates often exhibited subtypes with higher levels of similarity to isolates from birds of the same species or feeding guild, than to isolates from other groups of birds. Only two birds had subtypes that were similar to those of human strains. Chuma et al. (2000) demonstrated that sparrows, as birds strongly associated with human activities, can also be a potential source of contamination of poultry. A study from Germany estimating the prevalence of *Campylobacter* in wild pheasants demonstrated that approximately 26% may be positive (Atanassova and Ring, 1999). Heryford and Seys (2004) published data on occupational outbreaks of campylobacte-

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riosis associated with employees exposed to the pathogen at a pheasant farm.

The study described in this paper was performed to type *Campylobacter* isolates from wild and farmed pheasants, and provide information that could be used to assess their relative importance for human disease.

MATERIAL AND METHODS

At the turn of 2005 and 2006, cecal samples from 302 pheasants from two flocks from a farm with intensive production, and from 200 wild birds were collected. Samples were taken in two stages. In the first phase, 200 birds from a farm and 200 wild pheasants were examined. The farmed birds aged five months were bred in a house on deep bedding for six weeks and then moved to a roofed outdoors run. Wild pheasants came from a reproductive farm in the CR, they were released to open country aged eight weeks and were fed no commercial fodder in the open nature. Examined wild pheasants were hunted and killed during a three-day hunt in the woods of the same geographical area. In the second phase, 102 farmed birds, aged eight months, and bred identicaly as before were examined.

1 g of cecal intestinal content of each pheasant was diluted and spread on the surface of selective modified charcoal cefoperazone deoxycolate agar plates and cultured according to the CSN ISO 102 72 Standard.

Specific identification and differentiation of thermophilic *Camplyobacter* spp. was performed by the PCR/RFLP analysis of the variable part of the gene for 23S rRNA (Fermer and Engvall, 1999).

For *fla*-RFLP typing the variable part of the flagellin A gene of *Campylobacter jejuni* strains was amplified (Steinhauserova et al., 2002). Digestion of the PCR product 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.

Further typing of these *C. jejuni* strains was performed by PFGE analysis of the whole chromosome using restriction endonuclease *Sma*I. The DNA fragments were separated on CHEF-DR III system (BioRad) in 1% agarose in three steps at 200 V, angle 120°C and 9°C with pulse time of 5–10 s for 4 h, 10–40 s for 14 h and 50–60 s for 4 hours. PFGE results were evaluated using *Total*Lab software (Phoreticx).

RESULTS AND DISCUSSION

Campylobacter spp. were recovered from 502 samples from all pheasants (n = 302 farm/n = 200 wild) (Table 1). The prevalence of *Campylobacter* spp. in the intestinal content of pheasants from the farm was 70.20% (212 samples). 50.47% of isolates were identified as *C. coli* and 41.40% as *C. jejuni*. There was a no statistically significant difference between the prevalence of *C. coli* or *C. jejuni* in farmed pheasants ($P > 0.05$). There is little information about incidence of *Campylobacter* spp. in pheasants. The Russian study (Stern et al., 2004) mentions approximately 25% prevalence on pheasant farms. High occurrence is also presumed by Heryford and Seys (2004) in their study monitoring pheasant farming in the U.S.A., in view of the high incidence of campylobacteriosis (53%) in farm employees. Compared with the prevalence of thermotolerant strains of *Campylobacter* spp. in broiler chickens, where identical farming technologies and composition of fodder blends are used (Padungton and Kaneene, 2003), our results show a higher percentage of *C. coli* species in pheasants. In general, our study has found a high incidence of *Campylobacter* spp. in pheasants originating from farms with intensive production.

In samples from wild pheasants, *Campylobacter* spp. positivity was proven in only 27.5% of cases.

Table1. Prevalence of thermophilic *Campylobacter* spp. in pheasants

Origin	Total		*C. coli*		*C. jejuni*		*C. coli* + *C. jejuni*	
	n/*N*	%	*n*/*N*	%	*n*/*N*	%	*n*/*N*	%
Farmed	212/302	(70.20)	107/212	(50.47)	87/212	(41.04)	18/212	(8.49)
Wild	55/200	(27.50)	20/55	(36.36)	32/55	(58.18)	3/55	(5.46)

n/*N* = the number of positive samples/the number of tested samples

The difference in prevalence between farmed and wild birds could be due the fact that samples from wild pheasants were not taken immediately after they had been shot, while the samples from farmed pheasants were gathered within two hours after their death. *C. jejuni* was more prevalent species (58.18%) than *C. coli* (36.36%) in the wild birds and mixed infection was confirmed in 5.46% of examined animals (Table 1). Our results are similar to the study from Germany estimating the prevalence of *Campylobacter* spp. in wild pheasants. In that study the authors demonstrated that approximately 26% may be positive (Atanassova and Ring, 1999). Waldenstrom et al. (2002) monitored various wild birds and found that the mean prevalence of *Campylobacter* spp. infection was 21.6% for all tested birds, but differed significantly between ecological guilds of birds and seems to be linked to various phylogenetic factors. Chuma et al. (2000) found that the *C. jejuni*-positive rate from sparrows was 2.6%, but they consider that the real positive rate may be higher.

252 isolates of *Campylobacter* spp. (132 *C. jejuni*, 118 *C. coli* and two undetermined *Campylobacter* spp.) were subjected to PFGE, using *Sma*I restriction enzyme but only *C. jejuni* strains were classified as particular subtypes by *fla*-RFLP. 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. A total of 15 PFGE (A–N) subtypes were noted among the *Campylobacter* spp. isolates. Some of the patterns were quite similar with minor variations usually with bands absent or one size shifted band. Point mutations, deletions or insertions may account for such small differences in profiles within subtypes. The generally accepted interpretation rule is that isolates are closely related when the fragment difference is around two to three fragments (Tenover et al., 1995). *C. jejuni* clones could be divided into 16 *fla*-RFLP subtypes. Table 2 compares PFGE profiles occurrence in *C. jejuni* and *C. coli* with respect to the origin of the samples. The highest frequency was observed for subtypes A, B, C, D, G and I without significant differences for bacterial species. No PFGE profile specific for *C. jejuni* and *C. coli*, was found. PCR/RFLP subtyping was performed on 122 isolates which were identified as *C. jejuni* (Fermer and Engvall, 1999). 51 samples gave aberrant patterns after restriction enzyme digestion and were therefore regarded as nontypeable. As PCR/RFLP method (Nishimura et al., 1996) was designed for subtyping human strains, these particular strains may have not been detected in humans yet. The prevalence of PCR/RFLP subtypes of *C. jejuni* is shown in Table 3. 16 PCR/RFLP subtypes were found with only subtype 1 occurring at a higher

Table 2. Prevalence of the PFGE subtypes of *Campylobacter* spp. in pheasants

PFGE	*C. jejuni*			*C. coli*			Total
	farmed	wild	Σ	farmed	wild	Σ	
A	15	4	19	10	4	14	33
B	4	12	16	11	6	17	33
C	12	–	12	9	–	9	22*
D	4	2	6	14	–	14	20
E	5	2	7	1	1	2	9
F	–	5	5	1	9	10	15
G	13	–	13	8	–	8	21
H	2	–	1	–	1	–	3
CH	3	–	3	11	–	11	14
I	13	–	13	7	–	7	20
J	7	–	7	10	–	10	18*
K	8	4	12	6	1	7	19
L	6	–	6	2	–	2	8
M	7	–	7	4	–	4	11
N	4	–	4	2	–	2	6

*one species not identified

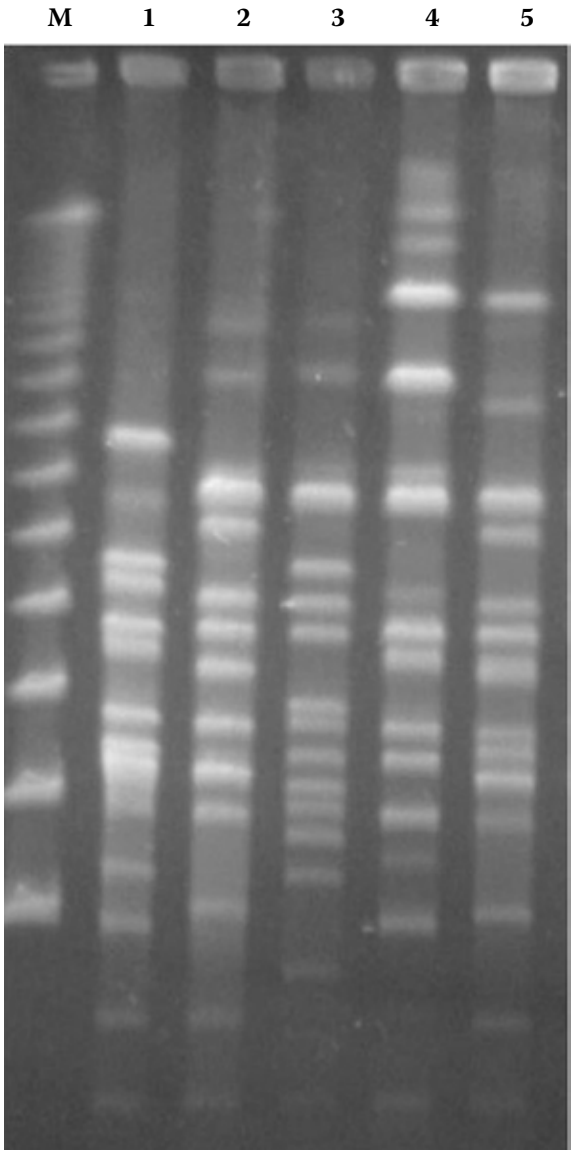

Figure 1. Various *Sma*I PFGE profiles of the same PCR/RFLP subtype (1)

M = molecular size marker; lane 1 = 1/E; lane 2 = 1/A; lane 3 = 1/I; lane 4 = 1/G; lane 5 = 1/K

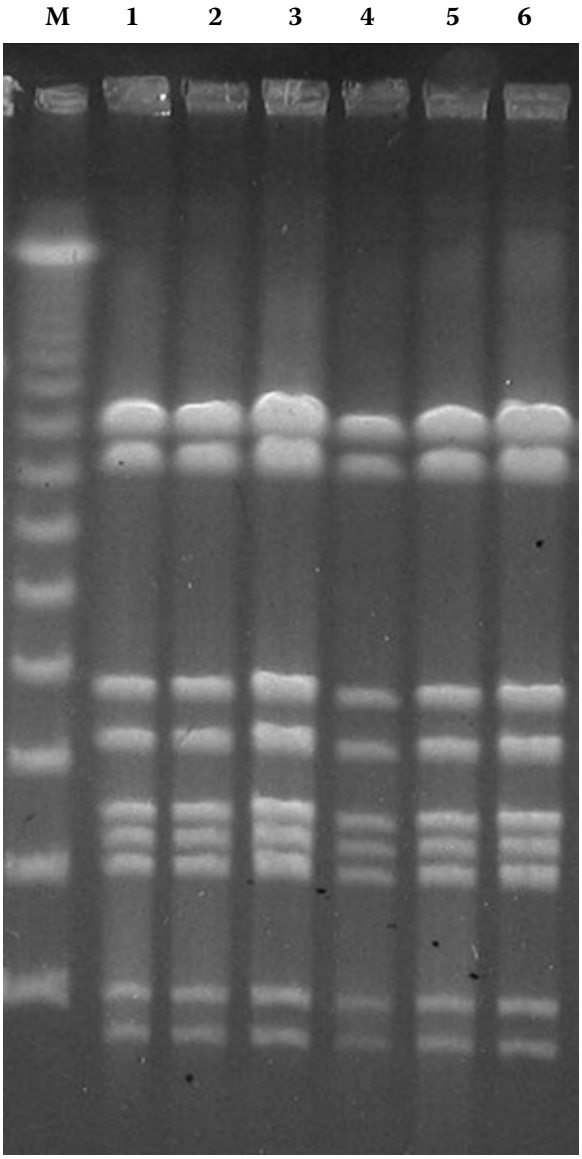

Figure 2. Various PCR/RFLP subtypes with the same *Sma*I PFGE profile (M)

M = molecular size marker; lane 1 = 1/M; lane 2 = 12/M; lane 3 = 14/M; lane 4 = 22/M; lane 5 = 23/M; lane 6 = 24/M

frequency and only in farmed pheasants. Similar to PFGE subtyping, the range of PCR/RFLP subtypes in wild birds is substantially limited, which might be not only due to a different way of life (farm birds are in closer contact which make the opportunity of mutual infection feasible) and diet but also due to contamination by farm workers or the relatively lower proportion of positive samples. As in PFGE analysis, not all different PCR/RFLP subtypes have to be non-related bacterial clones. Subtypes 3, 7, 9, 12, 18 and 23 can be derived from subclone 1 after a simple change of sequence (mutation) in the recognition site for one of the restriction enzymes used. For these reasons, the variability can be presumed to originate from an initial infection with a single clone. Genotypic diversity in a pheasant flock on the farm is similar to that of broiler flocks. *C. jejuni* is a species with genomic instability that affects flagellin typing (Wassenaar et al. 1995) and PFGE (Hanninen et al. 1999). On the other hand, many *C. jejuni* strains have been shown to remain genetically stable (Manning et al., 2001). For these reasons some authors describe common genotypes in groups of animals raised simultaneously on the

Table 3. Prevalence of the PCR/RFLP subtypes of *Campylobacter jejuni* in pheasants

PCR/RFLP	*C. jejuni*		
	farmed	wild	total
1	23	–	23
3	1	–	1
4	3	3	6
5	1	–	1
6	1	1	2
7	5	–	5
9	–	1	1
10	3	–	3
11	1	–	1
12	4	–	4
14	3	–	3
18	1	–	1
21	9	–	9
22	4	–	4
23	1	5	6
24	1	–	1

same farm (Guevremont et al., 2004), others have reported that broiler flocks can be colonized by 1–3 and parent flocks by 2–6 different *Campylobacter* clones. Rather than exclusive colonisation, clones coexist within one flock (Petersen et al., 2001). In our analysis, mixed contamination with two different clones was found on seven occasions.

Relationships among individual clones were also detected by combinations of subtypes identified by both methods. Clones displaying the same characteristics for both PCR/RFLP and PFGE subtypes can be considered closely related as opposed to clones that match in only one subtype (Figure 1 and 2). When comparing wild birds with farmed pheasants, it was determined that there are eight possible combinations of PCR/RFLP and PFGE subtypes in wild birds and 40 combinations in farmed pheasants. No preferred combination of both subtypes was detected, all combinations were randomly ordered. Only one combination 4/B was confirmed in both types of samples (on two occasions). Combinations 23/A, 10/B and 23/D were isolated twice in wild birds, whereas in farm pheasants 1/E (on five occasions) and 1/A, 7/C, 1/I, 14/J (on three occasions each) were detected with a higher frequency.

Our results inform the question of primary source of infection in the whole flock. Either the flock has already been the carrier of a single bacterial clone, or more clones are present and during the colonization the type with higher colonization potential is expanded. Experiments carried out by Korolik et al. (1998) have shown that all *C. coli* strains isolated from humans were able to colonize chicken intestines, while four out of seven *C. jejuni* strains evaluated failed to colonize. Furthermore, ten out of the twelve poultry *C. jejuni* strains tested were able to colonize chicken intestines permanently, while two strains colonized the intestines either poorly or not at all. Other reports contradict local transmission, which may support the hypothesis of association between different subtypes of *C. jejuni* and certain species or guilds of hosts (Broman et al. 2004).

To determine the significance of pheasants as a source of *C. jejuni* human infection, the RFLP and PFGE patterns of pheasant isolates are currently being compared with those of isolates from diarrheic patients.

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