DNA-FINGERPRINTING OF *CAMPYLOBACTER* SPP. FROM POULTRY AND GENETIC RELATIONSHIP OF SELECTED STRAINS TO HUMAN ISOLATES

Klein, G.[1], Beckmann, L.[2], Luber, P.[2] and Bartelt, E.[2]
[1]Institute of Food Quality and Safety, School of Veterinary Medicine, Bischofsholer Damm 15, D-30173 Hannover, Germany
[2]Federal Institute for Risk Assessment, Diedersdorfer Weg 1, D-12277 Berlin, Germany

Background

An important source of infection with *Campylobacter* is considered to be raw poultry meat. A lot of broiler flocks are infected with *Campylobacter* spp. (Newell & Wagenaar, 2000), in Germany about 41% (Atanassova & Ring, 1999). Chicken colonized with *Campylobacter* spp. excrete large amounts of bacteria (Stern et al., 1995). After colonized birds have entered the slaughterhouse processing lines *Campylobacter* can be found on the chicken carcasses throughout the slaughtering process and result in contamination of equipment, working surfaces, process water and air (Jacobs-Reitsma, 2000). On the other hand case-control studies have identified the handling and consumption of poultry as a major risk factor for *Campylobacter* infections. To evaluate the spread of *Campylobacter* through the slaughterline and possible genetic relationship between isolates from poultry products and human isolates DNA fingerprint techniques are needed. Macrorestriction analysis by pulsed-field gel electrophoresis (PFGE) has been used successfully for inter- and intraspecies differentiation of *Campylobacter* (Ribot et al., 2001) and PFGE is one of the most discriminatory genotypic typing methods currently available for subtyping *Campylobacter* species.

Objectives

The aim of this study was to differentiate isolates of *Campylobacter* spp. at various stages throughout processing with DNA fingerprinting techniques. Genotyping of the selected strains by PFGE should determine subtypes which were common for carcasses and offal and prove if cross-contamination within and between flocks occured. To assess the impact of poultry products as vectors for sporadic *Campylobacter* infections in Germany, in this study the relatedness of *Campylobacter* spp. strains occurring in a 3-month period within the city of Berlin on retail chicken products and in cases of human campylobacteriosis by pulsed-field gel electrophoresis (PFGE) were analysed.

Materials and methods

Eight poultry flocks from different farms were sampled at different dates: flock „a“ and „b“ in June, flock „c“ and „d“ in July,; flock „e“ in August, flock „f“,„g“ and „h“ in September, and flock „g“ in October. Cloacal swabs were taken at the beginning of the slaughter, and neck skin samples before evisceration were taken from each flock. Crates for living birds were sampled before and after washing, carcasses after scalding and neck skin after air spray chilling in some flocks, additionally.
Detection of thermophilic *Campylobacter* spp. was performed by selective enrichment in Preston-broth (Bolton, 1983) following isolation on Preston-agar (Oxoid, CM 67 plus selective supplements SR 117 + SR 48. Oxoid GmbH, Wesel, Germany). One presumptive *C.* isolate from each selective agar plate was identified to species level on the basis of phase-contrast microscopy (characteristic morphology and motility), Gram stain, catalase and oxidase production, growth at 25°C and 43°C, indoxyl acetate hydrolysis, hippurate hydrolysis, and susceptibility to nalidixic acid and cephalotin. All isolates were stored at -80°C in a freezer using the Microbank™ system (PRO-LAB Diagnostics, Cheshire, UK). *C. jejuni* type-strain ATCC 33560^T and *C coli* type-strain ATCC 33559^T were used as controls.
A subset of 154 *Campylobacter* spp. strains were choosen from each flock at each processing station for ribotyping in a previous study (Vollmer, 1996) and the same strains were collected for subtyping with pulsed-field gel electrophoresis.
For the evaluation of the genetic relationship between poultry product isolates and human isolates *Campylobacter* spp. strains were isolated in Berlin, Germany, from January to March 2002. 31 *C. jejuni* and 6 *C. coli* from chicken product samples and 40 *C. jejuni* and 7 *C. coli* from cases of human campylobacteriosis were subtyped.

Poultry food samples (chicken) were taken at retail level. Besides different meat samples (breasts, drumsticks, and wings), sampling included liver, gizzard and heart, presenting the broad range of chicken products consumed regularly by german consumers. Isolation of *C.* spp. from foods was performed in general accordance with the guideline ISO 10272 (International Organization for Standardisation, 1995). Human isolates originated from ambulant cases of diarrhea (stool samples) occurring in Berlin and have been isolated following standard laboratory methods.
Isolates were subtyped by PFGE using the restriction enzyme *Sma*I and *Kpn*I. Strains from poultry products and human isolates were only additionally analysed with with *Kpn*I when identical *Sma*I profiles were found. Preparation of DNA-containing agarose blocks for PFGE was adapted to a "CAMPYNET" prototype standard protocol (http.//campynet.vetinst.dk/PFGE.html) with following exceptions: 500mµl of cell suspension were mixed with 500µl warm 2% inCert-agarose (FMC BioProducts). DNA was cut for 18h with *Sma*I and *Kpn*I at appropriate temperature according to manufacturers instructions (New England Biolabs). Digested DNA plugs were loaded on a 1% SeaKem GTG agarose gel (FMC BioProducts). DNA fragments were separated on a CHEF DR-III apparatus (BioRad) in 0.5xTBE-buffer at 10°C for 22.5h (*Sma*I) or 23h (*Kpn*I). Ribotyping was performed and described in a previous study (Vollmer, 1996).
Computer analysis was carried out with GelCompar 4.1 (Applied Maths). Macrorestriction profiles were evaluated and assigned to arbitrarily-defined "profile groups", using a cut off of 90%.

Results and discussion

A number of genetically distinct *Campylobacter* strains were isolated at different sampling stations per flock. Twenty-one different ribotypes (RT), 24 *Sma*I macrorestriction profiles (MRP)-types and 25 different *Kpn*I MRP-types or profile groups (PGs) were identified. Regarding the distribution of types within the two *Campylobacter* spp. the 13 *C. coli* isolates yielded two different PFGE patterns (PFGE (*Sma*I / *Kpn*I), types X / X and Y / Y), and two different ribotypes (RT 14 and RT 16, respectively). 141 *C jejuni* isolates were divided into 22 distinct PFGE *Sma*I, 23 different PFGE *Kpn*I, and 19 distinct RT profiles, respectively. During the processing line at least one *Campylobacter* subtype appeared only in one flock, and was mainly isolated from cloacal swabs, e.g. PG P/P from flock „f", (Tab. 1). In nearly all flocks sampled, at least one additional *Campylobacter* subtype was recovered from the carcasses or offal at different processing steps compared to the subtypes isolated from cloacal swabs (Tab. 1). Some *Campylobacter* strains with identical profile groups were isolated from different flocks at different times. Strains with PG U / U (*Sma*I / *Kpn*I) were recovered in flock „b" and „h", and strains with PG C / C in flock „b" and „e"in June and September.
It is assumed that *Campylobacter* isolates on carcasses and offal with identical macrorestriction profiles (MRPs) like those detected in cloacal swabs were flock-specific and derived from cross-contamination within flocks during processing, e.g. defeathering or evisceration. The detection of farm-specific *Campylobacter* clonal isolates that occurred in successive rotations strongly indicates that those clones persist on the individual farms, either inside the broiler house or in the near environment (Petersen & Wedderkopp, 2001). Analysis of *Campylobacter* colonization of one flock during the rearing period and contamination of the carcasses at the slaughterhouse by *flaA* typing showed that for example in studies of Rivoal et al. (1999) seven of eight strains isolated from the chicken feces at the farm were also present at the slaughterhouse at neck skins taken directly after evisceration at the processing plant. In addition four other *fla*A-types were collected from these batch only at the slaughterhouse. These results confirm that contamination of broilers during processing was mainly due to the rupture of the gastrointestinal tract during evisceration (Rivoal et al., 1999) and to a lesser extent from other sources. In our study for example, in flock "d" we found only the MRP F and the RT 07 from cloacal swabs on carcasses and offal.
Genotyping by PFGE of poultry product isolates and human isolates identified 15 *Sma*I patterns that occurred in more than one isolate (n=50). As shown in table 2, the same *Sma*I pattern was found in up to 7 isolates. After the second analysis using the restriction enzyme *Kpn*I, 56 of the 84 isolates in total had a unique *Sma*I/*Kpn*I PFGE-profile, whilst 28 had a profile they shared with 1 to 5 other strains. The *Sma*I/ *Kpn*I -profiles III-6, V-12, and XI-24 encompassed human and poultry isolates. Two subtype groups with identical profiles were formed by strains of human origin only (VIII-19 and XIV-31) and 4 groups encompassed only strains of poultry origin (I-1, II-4, VI-15, XII-25). Genotyping by PFGE revealed a high degree of diversity, which is a well-known phenomenon for bacteria of the genus *Campylobacter*. But despite the weak clonality of *C.* spp., 10.6% of human isolates were genetically identical with isolates found in the same geographical region and time frame on retail chicken products.

Conclusions

The populations of *C. jejuni* and *C. coli* are extremely diverse and this diversity is refelcted in a broad spectrum of subtypes. Distinct flocks could be colonized with different types of *Campylobacter*. One flock could be colonized by multiple genotypes whereas strains within individual flocks coexisted rather than excluding each other. In addition more than one strain in each flock may have potential epidemiological importance. Concerning the possible genetic relationship of poultry product isolates and human isolates it can be concluded that retail chicken products are an important source for sporadic human infections with *C.* spp. in Germany.

References

Atanassova V., C. Ring. 1999. Prevalence of *Campylobacter* spp. in poultry and poultry meat in Germany. Int. J. Food Microbiol. 51: 187 - 190.

Jacobs-Reitsma, W. 2000. *Campylobacter* in the food supply, p. 467-481. *In* I. Nachamkin and M.J. Blaser (ed.), *Campylobacter*, 2nd ed. ASM Press, Washington D.C.

Newell, D.G. and J.A. Wagenaar. 2000. Poultry infections and their control at the farm level. p. 497-509. *In* I. Nachamkin and M.J. Blaser (ed.), *Campylobacter*, 2nd ed. ASM Press, Washington D.C.

Petersen L., A. Wedderkopp. 2001. Evidence that certain clones of *Campylobacter jejuni* persist during successive broiler flock rotations. Appl. Environ. Microbiol. 67: 2739 - 2745.

Ribot E.M., C. Fitzgerald, K. Kubota, B. Swaminatha, T.J. Barrett. 2001. Rapid pulsed-field gel electrophoresis protocol for subtyping of *Campylobacter jejuni*. J. Clin. Microbiol. 39: 1889-1894.

Rivoal K., M. Denis, G. Salvat, P. Colin, G. Ermel. 1999. Molecular characterization of the diversity of *Campylobacter* spp. isolates collected from a poultry slaughterhouse: analysis of cross-contamination. Lett. Appl. Microbiol. 29: 370-374.

Stern, N.J., M.R. Clavero, J.S. Bailey, N.A. Cox, M.C. Robach. 1995. *Campylobacter* spp. in broilers on the farm and after transport. Poult. Sci. 74: 937-941.

Vollmer, H.M. 1996 Untersuchungen zum Vorkommen, zur Verbreitung und Feindifferenzierung von *Campylobacter* ssp. in der Geflügelschlachtung. Thesis Vet.med., Free University of Berlin, Germany.

Tab. 1: PFGE profile groups (PG) and ribotypes (Vollmer, 1996) of isolates from selected flocks of poultry processing

	Flocks					
	F			G		
Point of sampling	Isolates (n)	PFGE-Grp. *Sma*I/*Kpn*I	Ribotype	Isolates (n)	PFGE-Grp. *Sma*I/*Kpn*I	Ribotype
transport crate / flock	2	I/I	6	nd		
transport crate before washing	not done (nd)			3	V/V	11
				1	G/G	12
transport crate after wash step	nd			5	V/V	11
				4	G/V	12
				1	S/S	13
cloacal swabs	1	P/P	19	1	V/V	11
carcass after scalding	nd			2	L/L	22
neck skin before evisceration	3	P/P	19	nd		
neck skin after chilling	5	P/P	19	2	V/V	11
	1	**Q/Q**	**19**			
offal swab	3	P/P	19	4	V/V	11
				1	G/G	12

Table 2: *Kpn*I-subtypes of 43 *C. jejuni* and 7 *C. coli* strains from poultry products and human origin which clustered in *Sma*I-profiles present in more than one isolate

Species	***Sma*I profile**	***Kpn*I profile**	**Origin of isolates**	**N isolates**
C. jejuni	I	1	chicken	2
C. jejuni	II	2	human	1
C. jejuni	II	3	chicken	1
C. jejuni	II	4	chicken	3
C. jejuni	III	5	human	1
C. jejuni	III	6	1 human 1 chicken	2
C. jejuni	III	7	human	1
C. jejuni	III	8	chicken	1
C. jejuni	IV	9	human	1
C. jejuni	IV	10	human	1
C. jejuni	IV	11	human	1
C. jejuni	V	12	2 human, 4 chicken	6
C. jejuni	V	13	human	1
C. jejuni	VI	14	human	1
C. jejuni	VI	15	chicken	2
C. jejuni	VII	16	human	1
C. jejuni	VII	17	chicken	1
C. jejuni	VIII	18	human	1
C. jejuni	VIII	19	human	2
C. jejuni	IX	20	human	1
C. jejuni	IX	21	human	1
C. jejuni	X	22	human	1
C. jejuni	X	23	human	1
C. jejuni	XI	24	2 human, 1 chicken	3
C. jejuni	XII	25	chicken	6
C. coli	XIII	26	chicken	1
C. coli	XIII	27	human	1
C. coli	XIV	28	chicken	1
C. coli	XIV	29	chicken	1
C. coli	XV	30	human	1
C. coli	XIV	31	human	2