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Original Contribution

DETECTION OF *CAMPYLOBACTER* USING STANDARD CULTURE AND PCR OF 16S rRNA GENE IN FRESHLY CHILLED POULTRY AND POULTRY PRODUCTS IN A SLAUGHTERHOUSE

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ABSTRACT

The present study provides data on contamination level with *Campylobacter* in poultry and poultry products after air chilling. Using cell culture and PCR we detected *Campylobacter* in the following proportion of chicken samples studied: 38.3% and 40.8%, respectively. *Campylobacter* contamination occurred highest in liver samples (53.3%), followed by the skin (46.6%), tight (36.6%) and breast (16.6%) samples in that order. *C. jejuni* (65.2%) was the most frequently isolated species, whereas *C. coli*, *C. fetus* and *C. upsaliensis* were isolated in 30.4%, 2.2%, and 2.2%., respectively. *C. jejuni ssp. doylei* (60%) was most commonly found subspecies of *C. jejuni*.

Key words: C *jejuni*, *C. coli*, liver, skin, poultry meat

INTRODUCTION

In recent years, the frequency of human enteritis caused by the *Campylobacter* has been on the increase in many developed countries (1, 2). Thermophilic *Campylobacter spp*., mainly *Campylobacter jejuni* and *Campylobacter coli*, have been recognized as a major cause of human gastroenteritis throughout the world. The potential source of infection has been linked to the consumption of undercooked poultry and poultry products contaminated with *Campylobacter* species (3)

Campylobacter spp. has been isolated from up to 82 % of broiler flocks at slaughter (4, 5). Cross-contamination at the abattoir is unavoidable and *Campylobacter* dissemination occurs during poultry processing, especially after evisceration due to rupture of the gastrointestinal tract (6). Poultry meat leaving the slaughterhouse is often *Campylobacter* contaminated (7, 8).

Campylobacter organisms are fastidious and slow growing with specific requirements in incubation conditions. Identification methods for *Campylobacter* have traditionally involved the use of selective culture media combined with biochemical tests such as hippurate hydrolysis, nitrate reduction, nalidixic acid susceptibility and indoxylacetate hydrolysis. While selective media are very useful for the initial isolation of *Campylobacter*, biochemical methods of identification are often tedious and may give ambiguous results. Thus laboratory diagnosis of *Campylobacter* is expensive, laborious and time consuming.

A molecular technique for screening of poultry flocks, in contrast, would be cheaper, faster and of great value in facilitating intervention strategies at farm and abattoir levels. In recent years, PCR has increasingly been applied in the detection and identification of *Campylobacter*. Numerous published results obtained with this method have shown greatly improved accuracy and sensitivity, associated with fast sample processing (9, 10).

The aim of this study was to determinate the presence of *Campylobacter* spp. in poultry and poultry products after air chilling using conventional culture technique and PCR.

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MATERIAL AND METHODS

Samples collection

The samples were collected from a slaughterhouse in Northern Germany from three different broiler flocks during processing. The air chilling system was used for carcass chilling in the slaughterhouse.

During evisceration liver samples were collected from 10 birds per flock and after air chilling, the same 10 chicken were removed from the processing line. All samples were placed into sterile plastic bags, stored at 4˚C, transported to the laboratory in chilled boxes and analyzed within 24 h. From each carcass skin, breast- and thigh muscle samples were removed aseptically and cultured in enrichment broth.

Culture conditions

Skin, meat and liver samples were added to Preston enrichment broth in dilution 1:10 and homogenized for 1 min using a Stomacher (*Seward, Stomacher 400, UK*). Preston enrichment broth containing Nutrient Broth Nr.2 (*Oxoid, CM 67, UK*), 5% (v/v) saponinlyzed horse blood (*Oxoid, SR 48, UK*) and Preston Campylobacter selective supplement (*Oxoid, SR 204, UK*).

All samples were incubated at 37˚C for 24 h followed by 24 h at 42˚C. Following enrichment culture samples were inoculated onto modified Campylobacter charcoal deoxycholate agar (*Oxoid, CM 739, UK*) supplemented with CCDA antibiotic supplement (*Oxoid, SR 155, UK*) and incubated under the same conditions.

A microaerobic atmosphere (5%O_2, 10%CO_2, 85%N_2) was obtained in anaerobic jar (*Becton Dickinson, 4360628*) by placing gas generating kit (*Oxoid, BR 56, UK*).

Strains were identified presumptively as *Campylobacter* spp. on the basis of catalase and oxidase reactions and motility under phase-contrast microscopy (*Zeiss, Axiolab*) and differentiated by API Campy® (*Bio Mérieux, 20800, France*).

DNA isolation

DNA from an enrichment broth and from a bacterial growth was extracted by phenol-chloroform method as described Sambrook et al. (11).

Primers

The genes coding for 16S rRNA were amplified by PCR with the following primers in the conserved regions within the 16S rRNA gene: forward primer, PLO6, 5′ - GGTTAAGTCCCGCAACGAGCCGC- 3′; reverse primer, CAMPC5, 5′ - GGCTGATCTACGATTACTAGCGAT- 3′.

PCR

The PCR were performed as previously described by Gardarelli-Leite (12) and Atanassova et al. (13). The PCR amplification was performed in a thermal cycler Gene Amp 2400 (Perkin Elmer). Samples were incubated for 2 min at 96˚C to denature target DNA and were cycled 30 times at 94˚C for 30 s., 50˚C for 30 s. and 72˚C for 1 min. The samples were then incubated at 72˚C grad for 10 min for final extension and were maintained at 4˚C until they were tested.

Electrophoresis

Amplified DNA was detected on a 2% agarose gel (Sigma, Deisenhofen) in 1x TBE buffer (Life Technilogies, Karlsruhe) at 90 V for 180 min. The gels were stained with ethidium bromide and photographed. The amplicons generated band ranged 283kb. size.

RESULTS

In an effort to detect the presence of *Campylobacter* spp. in poultry and poultry meat 120 skin, liver, breast- and tight meat samples were collected and subjected to both traditional culture techniques and PCR. The results are listed in **Table 1**. *Campylobacter* contamination was detected in 38.3% and in 40.8% of the samples when analyzed by the bacteriological and molecular technique, respectively. By use of PCR we found additional 3 samples (two of the skin samples and one of the liver samples) as *Campylobacter* positive (**Table 1**). PCR were performed with DNA extracted from an enrichment broth (**Fig 1**).

All of 46 obtained presumptively *Campylobacter* isolates were confirmed as *Campylobacter* by biochemical tests concluded in API Campy ® and by PCR performed from a bacterial growth (**Fig 2**).

Altogether, 46 of the 120 chicken skin, liver, breast- and tight meat samples (38.3%) investigated proved to be contaminated with *Campylobacter*. The liver samples had the highest contamination rate (53.3%), whereas breast meat (16.6%) was as nearly three times lower contamination as that for breast skin (46.6%) (**Table 1**). In 14 of the sampled

carcasses *Campylobacter* was detected in the skin samples, but not in the breast- and tight meat samples. *Campylobacter* was not isolated in only 4 livers of the same 14 birds.

Sample	n	*Campylobacter* positive			
		by cultural method		by PCR method	
		n	%	n	%
liver	30	16	53.3	17	56.7
skin	30	14	46.6	16	53.3
breast meat	30	5	16.6	5	16.7
thigh meat	30	11	36.6	11	36.7
Total	**120**	**46**	**38.3**	**49**	**40.8**

Table 1. Detection of *Campylobacter* in poultry samples by conventional culture method and by PCR amplification in gene coding for 16S rRNA.

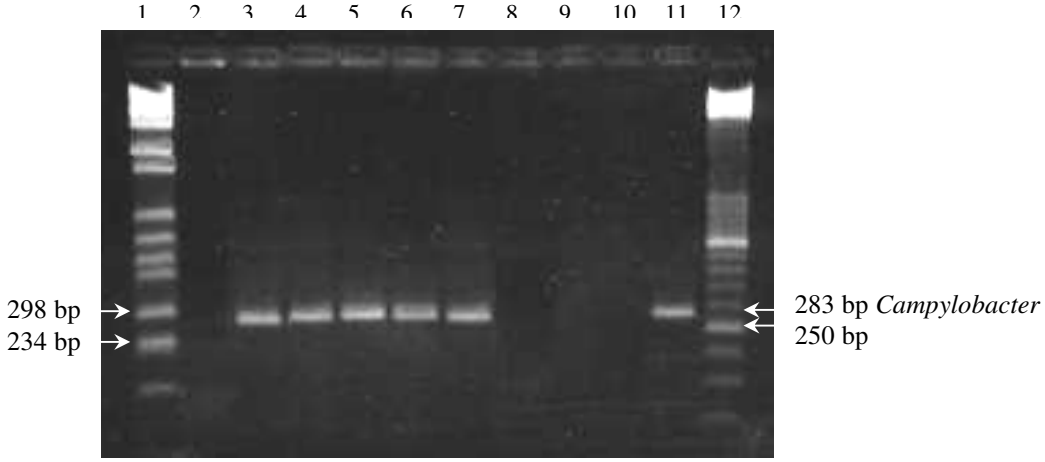

Fig. 1. PCR for direct detection of *Campylobacter* after enrichment of the samples in Preston enrichment broth. Lane 1, bp ladder VI; Lane 2, a negative control; Lane 3, a positive control (*C. jejuni* DSM 4688); Lanes 4 - 11, amplicons from Preston enrichment broth; Lane 12, bp Ladder XIII.

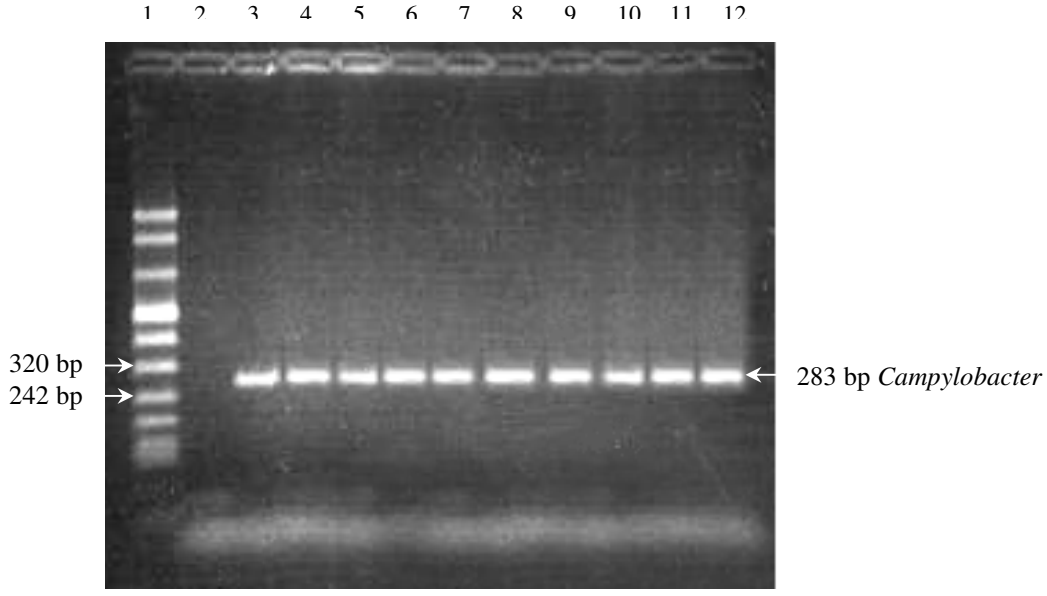

Fig. 2. PCR for identification of presumptively *Campylobacter* bacterial growth obtained on CCDA after enrichment step in Preston enrichment broth. All the samples yielded an amplicon size of 283 bp. and were confirmed as *Campylobacter* spp. Lane 1, bp ladder VIII; Lane 2, a negative control; Lane 3, a positive control (*C. jejuni* DSM 4688); Lanes 4 - 12, amplicons from bacterial growth on CCDA.

Samples / positive	*C. jejuni*		*C. coli*		*C. fetus*		*C. upsaliensis*	
	n	%	n	%	n	%	n	%
120 / 46	30	65.2	14	30.4	1	2.2	1	2.2

Table 2. Species differentiation of the *Campylobacter* isolates obtained in slaughterhouse.

Campylobacter jejuni	n	Liver	Skin	Breast meat	Thigh meat
		%	%	%	%
C. jejuni ssp. jejuni	12	50.0	16.7	16.7	16.7
C. jejuni ssp. doylei	18	33.3	38.9	11.1	16.7

Table 3. Subspecies differentiation of *C. jejuni* isolates detected in liver samples and in skin and muscle samples from chicken carcasses after air chilling.

The species identification showed that *C. jejuni* (65.2%) was the most frequently found followed by *C. coli* (30.4%), *C. fetus* (2.2%) and *C. upsaliensis* (2.2%) (**Table 2**). Percentage of *C. jejuni* was the highest in the liver (40%) while *C. coli* was most commonly detected in the tight meat samples (20%).

Table 3 contains the results of subspecies differentiation of all *C. jejuni* isolates obtained from the birds in slaughterhouse. The most frequently found subspecies was *C. jejuni ssp. doylei* (60%), which was detected with the highest percentage in the liver samples (50%), whereas *C. jejuni ssp. jejuni* was found at highest in the skin samples (38.9%).

DISCUSSION

Poultry and poultry products leaving the slaughterhouse are contaminated with *Campylobacter* (14). Stern et al. (15) reported *Campylobacter* in 62% of the carcasses after the chilling and observed level of contamination between $\log_{10}4.97$ and $\log_{10}2.95$ cells.

A lot of *Campylobacter* positive samples has also been detected after air carcass chilling (16). In our study *Campylobacter* was present in 46.6% of the skin samples after air chilling of the carcasses. Even though *Campylobacter* is highly sensitive to drying under laboratory conditions, it is likely that the chicken skin is providing an appropriate microenvironment to protect *Campylobacter* enabling them to survive successfully in the slaughterhouse on the carcass skin and in finished commercial poultry products.

The data from our investigation clearly demonstrate the high risk of acquiring food borne infection with *Campylobacter* when eating insufficiently cooked chicken flesh.

In our study the highest *Campylobacter* contamination rates were obtained with the liver samples (53.3%). Other authors found liver contamination in the ranges of 28.6% to 92.9% (17, 18). This result is important in food hygiene circle since it could lead to high risk of infection among consumers who might eat insufficiently-cooked chicken liver. In addition, the liver, if packed inside the carcasses, becomes a good vehicle for *Campylobacter* spread inside the body cavity as well as the chicken skin surface.

We found *C. jejuni ssp. doylei* (60%) as the most frequently isolated subspecies of *C. jejuni*, while *C. jejuni ssp. jejuni* was isolated in 40% of the assayed samples. Atabay et al. (19) determined *C. jejuni ssp. jejuni* in all studied samples, while other authors detected *C. jejuni ssp. jejuni* in only 10% of the poultry samples (7). *C. jejuni ssp. jejuni* is highly pathogenic and is the most commonly isolated *C. jejuni* subspecies from patients suffering gastrointestinal diseases, whereas the pathogenic potential of *C. jejuni ssp. doylei* is yet to be fully appreciated. This subspecies can be isolated from gastric biopsy specimen (20), from children's blood cultures (21) and from patients with diarrhea (22). All these suggest the pathogenic characteristics and invasive potential of *C. jejuni ssp. doylei*.

The results we found clearly show that the molecular and bacteriological methods are

equally reliable for detection of *Campylobacter* contamination of poultry and poultry products. We did not find any statistical significant difference between both methods in the determination of Campylobacter. However, PCR has many advantages compared to bacteriological method - rapid response and high sensitivity are some of them. By use of PCR we were able to detect Campylobacter in the samples within 2 - 3 days compared with the 4 - 5 days required to determine the presence of Campylobacter on agar plate Before DNA isolation enrichment of the samples was required and this reduced the time and the number of selective media for *Campylobacter* isolation.

The sensitivity of the PCR for detection of *Campylobacter* from an enrichment broth varied from 500 CFU ml^{-1} (18) to 10^2 - 10^3 CFU ml^{-1} (33).

The primers we used are suitable for *Campylobacter* screening, but not for species identification. Primers based on species specific gene sequences are described for further *Campylobacter* differentiation (15, 16). However it is still necessary to perform streaking on agar plates to recover isolates for further species identification by PCR.

CONCLUSION

The results from this investigation show that high percentage of the chilled poultry and poultry products leaving the slaughterhouses are *Campylobacter* contaminated. Poultry meat without skin also presents risk for consumer to acquire *Campylobacter* infection. Between the cultural and PCR technique for detection of *Campylobacter* there were no statistical significant difference, but the molecular method was as nearly two times faster than the traditional bacteriological method we used. These advantages of the PCR technique enhance its application as a short-time method of *Campylobacter* screening of poultry and poultry products.

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