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# The importance of *Campylobacter coli* in human campylobacteriosis: prevalence and genetic characterization

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## SUMMARY

The primary aim of this study was to detect and genotype *Campylobacter coli* strains from humans over a period of one year from November 2002 to October 2003. *Campylobacter* spp. were isolated from patients with symptoms of enteric infection. Amplified fragment length polymorphism (AFLP) analysis was used to identify the genetic diversity of *C. coli* strains by cluster analysis. A total of 18·6% of all *Campylobacter* isolates were identified as *C. coli*. These data show, that *C. coli* is indeed of importance to human campylobacter infections. Heterogeneous patterns were detectable among the human *C. coli* pool by AFLP analysis suggesting different sources of infection. A continuous seasonal shift of genotypes was detectable.

## INTRODUCTION

*Campylobacter* spp. are one of the very common causes of infectious gastroenteritis in humans. In Germany, 54 599 human cases were reported in 2004 [1]. The species *Campylobacter jejuni* is predominant, while *C. coli* is largely regarded as of minor importance in causing human disease [2–4]. Therefore, most research on human *Campylobacter* infection has been carried out on the species *C. jejuni*. Unfortunately, most case-control studies have not sought to differentiate between *Campylobacter* spp. and did not take into accounts that *C. coli* and *C. jejuni* differ in their aetiology [5, 6].

There are only a few reports about the true extent and health burden of *C. coli*-induced human campylobacteriosis [4]. For example, in the north-west of England 7% of *Campylobacter* isolates from enteritic patients were identified as *C. coli* [7]. In Germany, the national data reported from the Robert Koch-Institute show a percentage of *C. coli* in human campylobacterosis of 13·5% in 2002 and 12·2% in 2003 [8, 9]. Nonetheless, more data are needed to assess the true extent and relevance of *C. coli* infection in humans. There is also a lack of sufficient information about the sources of human *C. coli* infection. Some suspected risk factors for *C. coli* infections in humans are: foreign travel, drinking untreated water, direct contact with infected animals or the consumption of meat pies [10]. There is evidence, suggesting that risk factors for human infection by *C. coli* are different from those factors responsible for *C. jejuni* infections [6]. *C. coli* and *C. jejuni* differ significantly in their prevalences in animal species and various environmental sources [11–13].

In order to assess the importance of *C. coli*-induced campylobacteriosis in humans the primary aim of this study was to detect and evaluate the prevalence of *C. coli* in humans. The second aim was to compare frequencies of exposures between annual seasons,

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demography and sex of all human campylobacteriosis cases. To characterize the genetic relationship of human *C. coli* strains, amplified fragment length polymorphism (AFLP) analysis was used to compare the different genotypes to identify the genetic diversity of the *Campylobacter* strains, recovered from enteric patients within a limited geographical area in Germany, the states Thuringia and Saxony.

## MATERIALS AND METHODS

### Human strains

In our study, 861 human *Campylobacter* strains, isolated as 'thermophilic campylobacters' over a 1-year period from November 2002 to October 2003 were tested. The strains were obtained from two regional microbiological laboratories (Laboratory 1: Symbiomed GmbH Labor Jena, Germany; Laboratory 2: Labor Leipzig Reising-Ackermann, Germany). Strains were isolated from patients with symptoms of enteric infection. Information was included about the patients' age, sex and onset of clinical symptoms. Strains were submitted on selective media either as pure culture or mixed culture.

### Identification of *C. coli*

*Campylobacter* isolates were subcultured: up to three single colonies were subcultured onto plates of *Campylobacter* blood-free selective medium (modified charcoal cefoperazone deoxycholate agar, mCCDA, Oxoid, Wesel, Germany) and incubated microaerobically (Anaerocult C; VWR, Darmstadt, Germany) in anaerobic jars (VWR) at 37 °C for 48 h. For species identification, single colonies were further processed by DNA extraction using the DNA Genomic Purification kit (Amersham Biosciences, Freiburg, Germany). Identification was carried out by PCR assays. Specific primers for the genus *Campylobacter* and the species *C. coli* [14] and *C. jejuni* [15] were used. The date of onset of illness was used to specify the distribution of cases per month.

### Genotyping

After identification, *C. coli* isolates were genotyped. In this study AFLP analysis was used for genetic characterization of *C. coli*-DNA extracts from human strains as described previously [16]. Approximately 20 ng genomic DNA was digested with 5 U *Hin*dIII and 5 U *Hha*I. Enzyme-specific adapters were simultaneously ligated to the digested DNA samples for 2 h at 37 °C. A pre-selective PCR was performed by using the diluted restriction-ligation mixture and the adapter-specific primers *Hin*dIII and *Hha*I. After pre-selective amplification, the PCR products were diluted and a selective PCR amplification round was carried out by using *Hin*dIII and *Hha*I selective primers. Both selective primers were extended by an additional A nucleotide at the 3′-end. Amplified DNA fragments were electrophoresed horizontally on a 12·5% SDS Gel (ExcelGel DNA Analysis kit, Amersham Biosciences) on a Multiphor II apparatus (Amersham Biosciences). Gels were stained with silver (PlusOne DNA Silver Staining kit, Amersham Biosciences), visualized and documented on a ChemiImager 4400 (Alpha Innotech, San Leandro, CA, USA) with AlphaEase Software version 5.5 (Alpha Innotech).

### Analysis of AFLP patterns

Cluster analysis was carried out by using Phoretix 1D Advanced version 5.20 and Phoretix 1D Database version 2.00 software (both from Nonlinear Dynamics, Newcastle upon Tyne, UK). Only AFLP bands in the molecular size range of 34–501 bp were analysed. After normalization and background subtraction, the bands were matched by Rf (retardation factor) to a synthetic reference lane. The position tolerance for band matching was set at 350 with no increase. Lane similarity of normalized AFLP profiles was calculated by Dice coefficient correlation. For cluster analysis of AFLP profiles, the unweighed pair-group method using arithmetic averages (UPGMA) was used. Correlation levels were expressed as percentage of similarity. The genotypes resulting from AFLP profiling were clustered at a cut-off similarity value of $\geqslant 90\%$.

### Statistical analysis

Interactions between *C. coli*, *C. jejuni*, sex and season were examined by using Pearson's $\chi^2$ test. Comparisons between the number of *C. coli* and *C. jejuni* cases, sex and age were likewise performed by $\chi^2$ analysis. The results were evaluated according to the program for statistics SPSS version 10.0 (SPSS, Chicago, IL, USA). Differences in proportions were considered statistically significant at $P<0{\cdot}05$.

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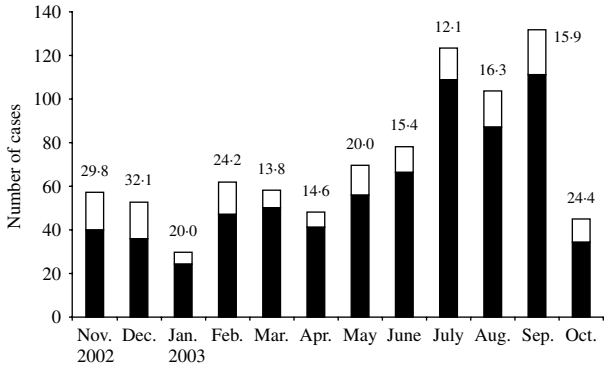


**Fig. 1.** Numbers of human campylobacteriosis cases, distribution between *C. coli* and *C. jejuni* reported in each month of the 1-year study. ■, *C. jejuni*; □, *C. coli*; number above each bar = percentage of *C. coli*.

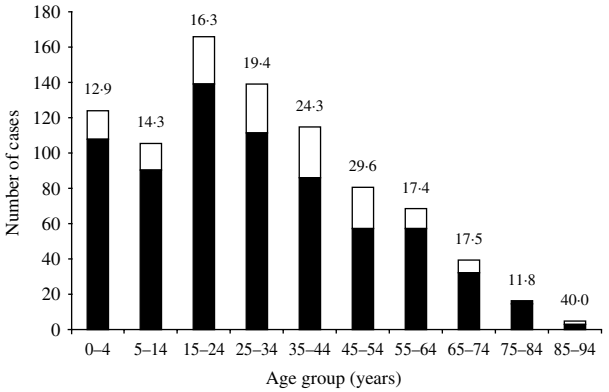


**Fig. 2.** Age distribution of *C. coli* and *C. jejuni* reported in the one year study. ■, *C. jejuni*; □, *C. coli*; number above each bar = percentage of *C. coli*.

## RESULTS

### Isolation rates and distribution of campylobacters

Of all 861 *Campylobacter* spp. strains submitted to us, 160 strains were identified as *C. coli* representing a *C. coli* prevalence of 18·6%.

Seasonal distribution of *Campylobacter* spp. demonstrates a low detection rate in winter and early spring (December to April) followed by an increase in cases in late spring (May and June), reaching the highest level in the summer month (from July to September). After the main peak in September, cases declined in autumn (October and November) (Fig. 1). Based on the total *Campylobacter* isolates, there were significantly more case patients with *C. coli* infection during the late autumn and winter months compared to the number of *C. coli* cases in the summer months ($P \leqslant 0{\cdot}001$) (Fig. 1).

Sex distribution showed no significant differences in the number of *Campylobacter* spp. cases between males and females (412 isolates *vs.* 449 isolates respectively). Accordingly, no differences in the sex distribution of *C. coli* cases were observed (males, 78 isolates; females, 82 isolates) (data not shown).

Age distribution of *C. coli* and *C. jejuni* cases is demonstrated in Figure 2, representing a bimodal distribution. Patients age ranged from $<1$ year to 85 years. The highest numbers of *Campylobacter* spp. cases were identified in the 15–24 years age group. A second peak was observed in the group of small children (0–5 years old).

### Cluster analysis of *C. coli* AFLP patterns

All of the 160 *C. coli* isolates were typed by the AFLP method. Using both enzymes *Hin*dIII and *Hha*I together with selective primers, we obtained highly reproducible band patterns. Cluster analysis showed a large number of genotypes that were highly heterogeneous: 30 different *C. coli* clusters were identified (at a $\geqslant 90\%$ cut-off similarity level). A continuous seasonal shift of genotypes was detectable throughout the whole sampling period, exemplarily demonstrated for two months (December 2002 and October 2003) in Figure 3. Details of these strains are given in the Table. Strains isolated in December 2002 were largely grouped in different clusters compared to those strains obtained in October 2003. Nonetheless single similarities between human strains could be established especially during the same sampling period (i.e. the same month of onset of illness). No specific regional patterns of *C. coli* genotypes were detectable by comparing strains submitted to us from the two different laboratories, which isolated strains from different regions of Germany.

## DISCUSSION

The objective of this study was to collect epidemiological data for *C. coli*, particularly with regard to human *C. coli* infections in selected regions of Germany over a 1-year period and to link this information to the AFLP genotype.

Other studies have previously described that within the genus *Campylobacter*, the species *C. jejuni* makes the greatest contribution to human disease, while *C. coli* is only responsible for a minority of human *Campylobacter* infections [3, 17]. Our findings demonstrate, that over the year tested, 18·6% of human campylobacteriosis were caused by *C. coli*. This prevalence in humans is higher than the official data

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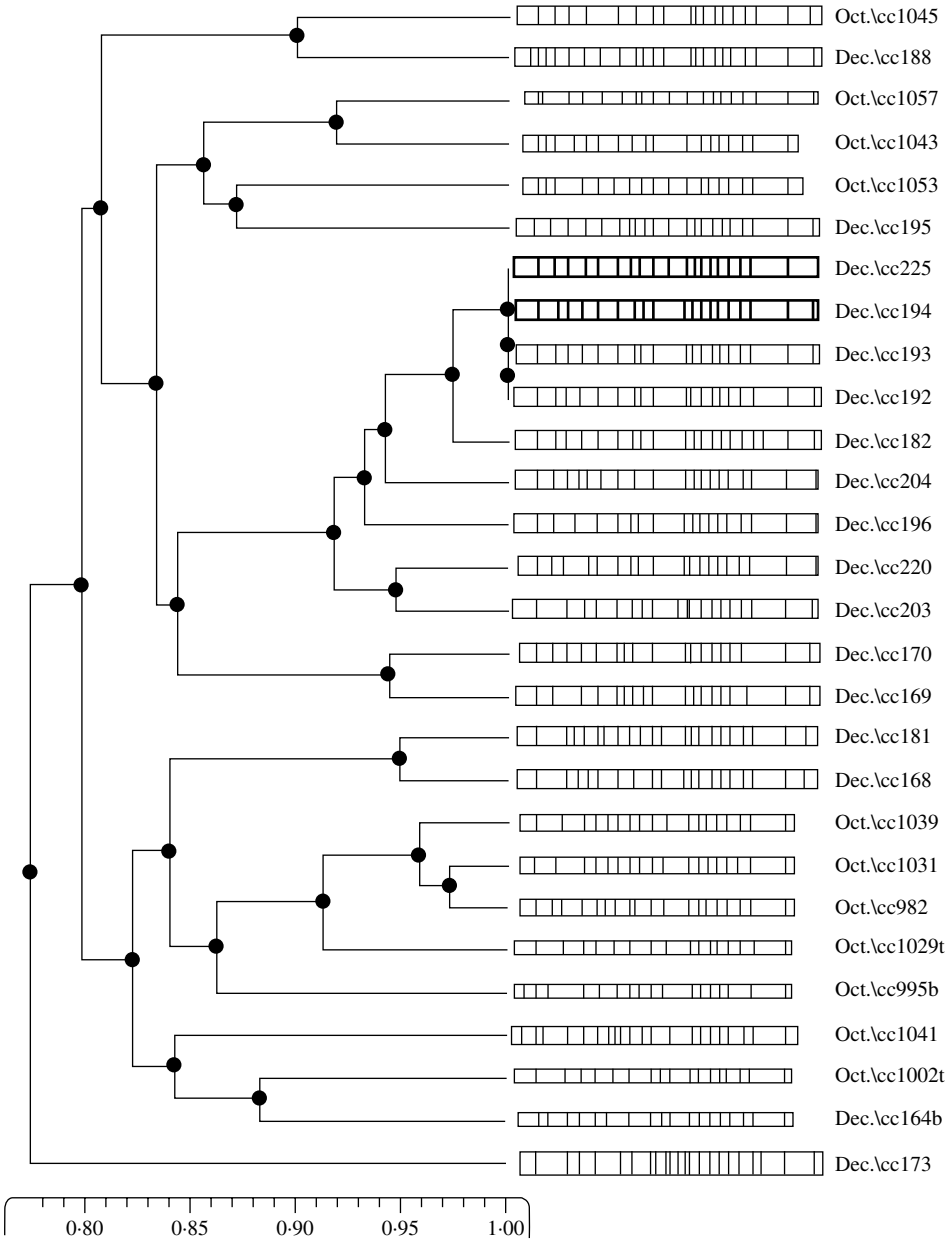


**Fig. 3.** UPGMA dendrogram of *C. coli* strains isolated in 2 months (December 2002, October 2003). The horizontal scale indicates % similarity between profiles.

for Germany reported by the Robert Koch-Institute [8, 9] and data generated in other countries [7, 18]. These discrepancies might be based on different identification methods. As Payot et al. [19] critically mentioned earlier, biochemical species verification tends to overestimate the *C. jejuni* involvement.

In our study, the bimodal age distribution of *Campylobacter* infections is comparable to data collected from Sopwith et al. [7]. They reported the highest numbers of *Campylobacter* spp. cases in the 25–34 years age group. In our study the highest numbers were found in the 15–24 years age group which is consistent with that observed in the 25–34 years age group. As suggested by Stafford et al. [20], the increased isolation rate in young adults might be caused by the higher travel frequencies of that age group. Data from typhoid infections support this argument: typhoid incidences are highest in the 15–29 years age group as well, and most of these infections are known to be acquired during travel [21]. The comparably high rate of infection in the $<4$ years old group was observed in other studies previously [20].

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Table. *Sources of* C. coli *strains isolated in December 2002 and October 2003*

| Strain ID | Lab. | Month of isolation | Age* | Sex† |
|---|---|---|---|---|
| cc164b | 2 | Dec. 2002 | 17 | f |
| cc 168 | 1 | Dec. 2002 | 3 | f |
| cc 169 | 1 | Dec. 2002 | 75 | f |
| cc 170 | 1 | Dec. 2002 | 67 | f |
| cc 173 | 1 | Dec. 2002 | 37 | f |
| cc 181 | 1 | Dec. 2002 | 27 | f |
| cc 182 | 1 | Dec. 2002 | 23 | m |
| cc 188 | 1 | Dec. 2002 | 3 | m |
| cc 192 | 1 | Dec. 2002 | 53 | m |
| cc 193 | 1 | Dec. 2002 | 53 | m |
| cc 194 | 1 | Dec. 2002 | 30 | m |
| cc 195 | 1 | Dec. 2002 | 21 | f |
| cc 196 | 1 | Dec. 2002 | 16 | f |
| cc 203 | 2 | Dec. 2002 | 40 | m |
| cc 204 | 2 | Dec. 2002 | 20 | m |
| cc 220 | 2 | Dec. 2002 | 26 | m |
| cc 225 | 2 | Dec. 2002 | 26 | f |
| cc 982 | 1 | Oct. 2003 | 30 | m |
| cc 995b | 1 | Oct. 2003 | 26 | m |
| cc 1002b | 2 | Oct. 2003 | 39 | m |
| cc 1029b | 2 | Oct. 2003 | 64 | f |
| cc 1031 | 2 | Oct. 2003 | 5 | f |
| cc 1039 | 1 | Oct. 2003 | 37 | m |
| cc 1041 | 1 | Oct. 2003 | 19 | f |
| cc 1043 | 1 | Oct. 2003 | 41 | f |
| cc 1045 | 1 | Oct. 2003 | 20 | f |
| cc 1053 | 1 | Oct. 2003 | 27 | f |
| cc 1057 | 1 | Oct. 2003 | 3 | m |

* Age of patient at the time of onset of illness.
† Sex of the patient: m, male; f, female.

Animal contact (owning a pet puppy or pet bird) and consumption of bird-pecked milk were described as specific risk factors in infants [22, 23]. But it may also reflect an over-sampling of this age group leading to higher numbers being recovered in the infant population [7].

No significant difference between the number of *Campylobacter* (*C. jejuni* as well as *C. coli*) infections in males and females was observed. Similar results were obtained by Gillespie et al. [6, 24]. In contrast to these results, Friedman et al. [5] demonstrated, that males are at increased risk for *Campylobacter* infection. But these authors as well as Louis et al. [25] could not identify major sex-specific differences in risk factors.

The seasonal distribution of *C. jejuni* infections shows an increase in May and a main peak in the summer month (July to September). This seasonal pattern is similar to that described in other studies [26, 27]. It has been suggested that the summer peak of *C. jejuni* may be due to the particular climatic conditions and the subsequent survival of *Campylobacter* spp. in environmental reservoirs [28], the corresponding seasonality in the number of animals infected [29] or be caused by an increase in foreign travel during the summer months, leading to a higher risk of imported infections [6]. The seasonality could additionally be explained by specific seasonal consumption of food (e.g. barbecues or consumption of unpasteurized milk) [30], seasonal changes in human immune parameters [31] and seasonal sports activities [7, 32].

In contrast to human *C. jejuni* infection, a winter peak of human *C. coli* cases was detectable in our study. Gillespie et al. [6] reported similar data, where *C. coli* infection was observed to be less likely in the summer season. Thus far, the reasons for that particular seasonality have not been explained. Different studies have attempted to link *Campylobacter* carriage rates in livestock to the seasonality of human *Campylobacter* infections [28, 29]. The *Campylobacter* carriage peaks in late spring and summer in broiler flocks and dairy cattle are both regarded as major sources of *C. jejuni* infection. In contrast, *C. coli* infection in pig herds appears not to be seasonally related [11]. Pork and pork products (such as pâtés and meat pies) may contribute to *C. coli* infections as proposed by Gillespie et al. [6] and Alter et al. [33]. In a large case-control study, Friedman et al. [5] identified the consumption of non-poultry meats (such as hamburgers, pork roasts and sausages) as an important risk factor for sporadic *Campylobacter* infections. It needs to be supported by further studies, if the consumption of minced pork meat, a typical German food, contributes to the high detection rate of *C. coli* in our localized survey.

To identify the genetic diversity of *C. coli* strains, particularly with regard to seasonal clones, we used AFLP analysis. AFLP, a whole genome fingerprinting method, has been used successfully for high-resolution genotyping of several bacteria including campylobacters [16, 34–36]. AFLP analysis revealed a large variety of genotypes among the *C. coli* pool, suggesting a large number of sources of infection. Despite that broad diversity of strains, single clonalities among strains that are possibly epidemiologically related, were identified (Fig. 3). These strains were either directly epidemiologically related (strain cc192 and cc193 were from the same patient at different sampling times) or were recovered at the same time

(strains cc194 and cc225 were submitted to the two different laboratories on the same day) suggesting similar sources of infection.

Our study suggests that the importance of *C. coli* as a human enteropathogen is largely underestimated. Further research is needed to establish the true extent of the public health implications of human *C. coli* infection. More data are required to detect the sources and the mode of transmission of that infection to humans. When adding the data presented here to the results of Tam et al. [4], who suggested that risk factors for transmission of *C. coli* differ to those for *C. jejuni*, different strategies will be needed to control both species.

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