Campylobacteriosis is the most commonly reported enteric bacterial pathogen in humans. We still do not have any systematic data concerning campylobacteriosis in Bulgaria. For that reason, we present data of the thermophilic *Campylobacter jejuni* and *Campylobacter coli* in the aetiology of diarrhoeal diseases in Sofia, for the period from 1987 to 2008. The study included patients from 0 to over 65 years old. A total of 51,607 faecal specimens were screened for *Campylobacter*. *C. jejuni* and *C. coli* were detected in 3.58% (1,847) of the strains, with the highest percentage in 1988 (7.5%) and the lowest in 2006 (0.3%). Campylobacteriosis occurred most frequently in the wet months of March, April, May and June, with 105, 102, 124 and 141 cases, respectively, and was rare in January with 25 cases. The most affected groups were children between 0 and 4 years of age (52%) and between five and 14 years of age (30%). *Campylobacter* infection occurred in 22% of all bacterial gastrointestinal diseases in the city of Sofia during the study period. *Salmonella* was the most frequently identified pathogen with 32%, followed by *Shigella* (30%), *Campylobacter* (22%) and diarrhoeagenic *Escherichia coli* (16%). The study shows that *Campylobacter* plays an important role as a bacterial cause of enterocolitis in Sofia, Bulgaria.

**Methods**

The study covered a period from 1987 till 2008 in Sofia, Bulgaria. Sofia has a population of about 1.5 million inhabitants. A total of 51,607 faecal specimens obtained from patients with enterocolitis were investigated for *Campylobacter, Salmonella, Shigella* and diarrhoeagenic *Escherichia coli*, i.e. enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC) and enterohaemorrhagic (EHEC) *E. coli*. Data were provided from the department of epidemiology at the National Centre of Infectious and Parasitic Diseases (NCIPD), Sofia, based on isolations of these bacterial pathogens by the Regional Inspectorate of Public Health Protection and Control, Sofia, and by five hospital and private laboratories in Sofia. The age of the patients ranged from 0 to over 65 years.

**Culture media**

The laboratory methods for *Salmonella, Shigella* and diarrhoeagenic *E.coli* were done according to the national standard method for diagnosis of enteric bacteria [4]. Faecal specimens for *Campylobacter* were

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**Figure 1**

Percentage of bacterial enteropathogens isolated from 51,607 faecal samples collected in Sofia, Bulgaria from 1987 to 2008

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>5.17%</td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td>4.93%</td>
</tr>
<tr>
<td>Diarrhoeagenic <em>E.coli</em></td>
<td>2.68%</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>3.58%</td>
</tr>
</tbody>
</table>

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**Introduction**

Campylobacteriosis is an infectious disease caused by thermophilic members of the bacterial genus *Campylobacter*. *C. jejuni* and *C. coli* are among the most important enteropathogens that cause gastroenterocolitis. The rate of *Campylobacter* infections worldwide is increasing, with the number of cases often exceeding those of salmonellosis and shigellosis [1,2]. These reported numbers of campylobacteriosis in many countries have revealed that this infection is emerging and becoming a major public health problem. According to the World Health Organization (WHO) *Campylobacter* is one of the most frequently isolated bacteria from stools of infants with diarrhoea in developing countries [3]. Despite the fact that campylobacteriosis is a notifiable disease in Bulgaria, there is no systematic data concerning this infection. In this report, we present data on the role of *C. jejuni* and *C. coli* compared to the other bacterial agents of diarrhoeal diseases in Sofia, Bulgaria.
inoculated on selective media containing 10% defibrinated sheep blood agar with Campylobacter selective supplement (BUL BIO-NCIPD, Bulgaria) and five antibiotics (vancomycin, trimethoprim, cefalotin, rifampicin and nystatin). The inoculated selective media were incubated for 48 hours in microaerophilic atmosphere with 10% CO₂ and 5-8% O₂, generated from packages Helico-Campy Pack (BUL-NCIPD, Bulgaria).

**Results**

**Isolates**

From the 51,607 investigated stool specimens, 1,847 isolates of Campylobacter (3.58%) were obtained. Of these, 75% were C. jejuni, 22% were C. coli and 3% belonged to other species. Salmonella was isolated most frequently, from 5.17% of the samples, followed by Shigella (4.93%), Campylobacter (3.58%) and diarrhoeagenic E. coli (2.68%) (Figure 1).

For the period of the study, Campylobacter infection occurred in 22% of all the bacterial gastrointestinal diseases in the city of Sofia. Salmonella was the most frequently isolated pathogen with 32%, followed by Shigella (30%), Campylobacter (22%) and diarrhoeagenic E.coli (16%) (Figure 2).

**Figure 2**

Distribution of the pathogenic enteric bacteria isolated from faecal samples collected in Sofia, Bulgaria from 1987 to 2008 (n=8,396)

**Table**

Proportion of pathogenic enteric bacteria isolated from 30,033 faecal samples in Sofia, Bulgaria, 1987-1997 (n=4,235)

<table>
<thead>
<tr>
<th>Year</th>
<th>Campylobacter (%)</th>
<th>Salmonella (%)</th>
<th>Shigella (%)</th>
<th>Diarrhoeagenic E. coli (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>6.20</td>
<td>4.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>1988</td>
<td>7.51</td>
<td>4.95</td>
<td>2.66</td>
<td>2.17</td>
</tr>
<tr>
<td>1989</td>
<td>5.00</td>
<td>4.55</td>
<td>0.67</td>
<td>2.67</td>
</tr>
<tr>
<td>1990</td>
<td>5.00</td>
<td>3.30</td>
<td>2.08</td>
<td>1.20</td>
</tr>
<tr>
<td>1991</td>
<td>2.47</td>
<td>3.10</td>
<td>3.13</td>
<td>2.37</td>
</tr>
<tr>
<td>1992</td>
<td>3.30</td>
<td>3.10</td>
<td>3.30</td>
<td>4.40</td>
</tr>
<tr>
<td>1993</td>
<td>6.17</td>
<td>2.61</td>
<td>3.00</td>
<td>3.30</td>
</tr>
<tr>
<td>1994</td>
<td>1.66</td>
<td>2.04</td>
<td>11.80</td>
<td>2.87</td>
</tr>
<tr>
<td>1995</td>
<td>5.17</td>
<td>2.19</td>
<td>6.55</td>
<td>1.75</td>
</tr>
<tr>
<td>1996</td>
<td>5.54</td>
<td>2.33</td>
<td>2.37</td>
<td>1.98</td>
</tr>
<tr>
<td>1997</td>
<td>6.49</td>
<td>1.59</td>
<td>1.70</td>
<td>2.90</td>
</tr>
<tr>
<td>Average (%)</td>
<td>4.95</td>
<td>3.07</td>
<td>3.57</td>
<td>2.51</td>
</tr>
</tbody>
</table>
Although *Salmonella* was on average the predominant enteric pathogen during the study period as a whole, *Campylobacter* predominated in the years 1987 (6.42%), 1988 (7.61%), 1989 (5.00%), 1990 (5.00%), 1993 (6.17%), 1996 (5.54%), 1997 (6.49%), 1999 (4.20%), 2000 (2.70%) and 2001 (4.90%). The highest proportion of *Campylobacter* was found in 1988 (7.50%) and the lowest in 2006 (0.30%).

In our previous study of 30,033 faecal specimens from the patients with enterocolitis in the period from 1987 to 1997, *Campylobacter* ranked first (4.95%) among the bacterial causes of enterocolitis [5], followed by *Shigella* (3.57%), *Salmonella* (3.07%) and diarrhoeagenic *E. coli* (2.51%) (Table).

### Seasonal distribution

The peak of *Campylobacter* infection in our study was in the wet months of spring and summer: on average 105 cases in March, 102 cases in April, 124 cases in May and 141 cases in June.

### Age distribution

An analysis of the age-specific incidence (Figure 3) showed that children up to the age of four years were the age group most affected by campylobacteriosis in Sofia (52%), followed by the group of 5-14-year-olds (30%), the group above the age of 65 years (6%), the 15-24-year-olds (5%), the 45-64-year-olds (4%) and the 25-44-year-olds (3%). In our study, *C. jejuni* and *C. coli* were most frequently isolated in the children up to the age of 14 years, totalling 82%.

### Discussion

Diarrhoeal diseases are a major problem for many countries in the world. The determination of the aetiological agent is an important step in the prophylaxis and the prompt treatment of enterocolitic infections.

In our study of 51,607 stool specimens, *Salmonella* was isolated most frequently, which correlates with reports of increasing incidence of human salmonellosis in Europe and the United States (US) in recent years [1,5]. The distribution of the different enteropathogenic bacteria among the positive faecal samples in our study was also similar to that observed in the US, where *Campylobacter* was isolated from 4.4%, *Salmonella* in 2.3% and *Shigella* in 0.9% of faecal samples in the same time period [7]. Campylobacteriosis was the leading cause of bacterial gastroenteritis reported in Belgium, Canada, Finland, Sweden, Central and South America, and southern states of Australia [8-10] during the time of our study.

In our study, *C. jejuni* and *C. coli* were most frequently isolated in the children up to the age of 14 years, totalling 82%. These data correlate with findings of other authors [1,10].

The diagnosis of *Campylobacter* in Sofia for the second decade in the study period, 1998-2008, was limited due to a shortage of data from hospital and private laboratories where the investigations are episodic.

*Campylobacter* enteritis has no seasonal preference in developing countries. In contrast, epidemics occur in summer and autumn in developed countries [1,11]. According to other authors in countries with moderate climate such as Bulgaria, *Campylobacter* is isolated most frequently in May, June and July [1,5], while the peak of *Campylobacter* infection in our study was in the wet months of spring and summer.

Our study provides data only for one region of Bulgaria, Sofia, although campylobacteriosis is notifiable disease in Bulgaria. The study showed the importance of thermophilic *Campylobacter* as a food-borne pathogen.
and underlines the need to strengthen surveillance of *Campylobacter* in Bulgaria. A lot of effort is needed to improve surveillance of campylobacteriosis in our country. Only a small number of laboratories are currently reporting *Campylobacter* cases. The main reason of the underreporting of campylobacteriosis in Bulgaria is the limited laboratory capacity for *Campylobacter* detection. The National Centre of Infectious and Parasitic Diseases in Sofia provides training in practical and theoretical courses on the diagnosis, treatment and epidemiology of campylobacteriosis. *Campylobacter* should be included in the set of enteric pathogens (*Salmonella*, *Shigella*, diarrhoegenic *E.coli*, *Yersinia*) tested for in cases of diarrhoea.

In conclusion, the results of our investigation for the period of 1987–2008 show that *Campylobacter* plays an important role as a bacterial pathogen that causes enterocolitis in Sofia, Bulgaria. The most affected group were 0-14-year-old children. Despite the fact that campylobacteriosis is a notifiable disease, the investigations are episodic and there is no systematic data for our country. For that reason we consider it an urgent need to introduce systematic surveillance of this infection in Bulgaria.

References