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Original scientific paper

Epidemiologic Characteristics of Human Campylobacteriosis in the County Primorsko-goranska (Croatia), 2003–2007

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A B S T R A C T

The aim of the study was to investigate campylobacteriosis incidence in the County Primorsko-goranska (Croatia) between 2003 and 2007 and to find out possible connection with environmental factors (the average monthly temperature and total monthly precipitation). The data (number of stool samples examined, age and sex distribution of patients, monthly distribution of isolates and distribution of isolates according to the species) from the Laboratory for Diagnostics of Enteric Infections of the Teaching Institute of Public Health of the County Primorsko-goranska (Croatia) were analyzed retrospectively. During the observed period 30,164 stool samples were examined for Campylobacter *spp.* Campylobacters *were identified in 1,242 (4.12%) samples. The overall annual campylobacter incidence rate was 81.3±21.9/100,000 population.* Campylobacter jejuni *was found in 1,093 (88%) and* C. coli *in 149 (12%) patients. Our findings showed age distribution of patients typical for developed countries. The patients were mostly children under 5 years (484.4±129.1/100,000, $p<0.001$) and between 5 and 9 years of age (226.5±60.5/100,000, $p<0.05$). Male consistently experienced higher rates, but the difference between genders was significant in the age groups from birth till late twentieth ($p<0.001$).* Campylobacter *rates were significantly associated with monthly average temperatures ($p<0.05$), but not with precipitation. Further investigations into the incidence of campylobacteriosis on the national level are necessary. The causes of the noticed monthly distribution, sources of infection and connection with the routes of transmission in humans need to be elucidated as well.*

Key words: *human campylobacteriosis, incidence rate, age, gender, month, precipitation, temperature, Croatia*

Introduction

Campylobacteriosis in humans is world wide spread gastrointestinal infection which is mostly presented in the form of acute enteritis. *Campylobacter jejuni* and *C. coli* are the most commonly isolated species from the stools of the patients with acute diarrhoeal disease (ADD)[1,2]. Poultry is regarded as the most important infection source for campylobacteriosis although consumption of barbecued meats, untreated water, unpasteurized milk, and contact with pets can be at risk also[3,4]. According to the reports campylobacteriosis is the most common of bacterial food borne infections in the community all over Europe. On the contrary in the USA salmonella still dominantes[5–8].

In temperate countries, a marked seasonal variation of human campylobacteriosis is seen, with the most cases occurring during the warmer season. It has been showed that in the northern European countries (Denmark, Netherlands) late summer peaks were present in comparison with the south of the Europe (Greece, Malta, Spain) where late winter or spring peaks were noticed[9]. On the contrary, on the Caribbean island of Barbados only small variations in the isolation frequency were observed, probably because of the tropical climate with no large temperature or rainfall oscillations[10].

The epidemiological data show a marked difference between developing and developed countries in the age

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distribution of patients with campylobacter caused ADD. In developing countries campylobacter infections are particularly common in very young children, under 2 years. Different age distribution is seen in developed countries where most infections occur in older children and young adults[11].

In spite of the numerous reports of the increasing incidence of human campylobacteriosis all over the Europe, there is no available epidemiological study for Croatia. There are no reliable data on the national level because campylobacteriosis was not reportable to public health authorities and there was no surveillance programme for campylobacteriosis. In July 2007 new law regulation was introduced and in accordance with EU (European Union) rules of disease monitoring, campylobacteriosis should be reported to the regional and national epidemiological services as a separate entity. That will enable analyzing the data concerning campylobacteriosis for each County and for the whole Croatia. For now, the resources on campylobacteriosis in Croatia are limited to rare published data, mainly from Zagreb area (northern, continental part of Croatia). We decided to analyze the available data for the County Primorsko-goranska (mainly littoral and island region). There were no published data about campylobacteriosis for this part of Croatia and according to the literature differences between regions in the same country can be found[12,13]. Public health laboratory for diagnostic of enteric infections in the County Primorsko-goranska is very well organized and for many years has been dealing with isolation and identification of campylobacters, the archives are well conducted and available. The aim of this study was to investigate the frequency of campylobacters isolated from stools of the outpatients with diarrhoea in the County Primorsko-goranska (Croatia) between 2003 and 2007. Number of stool samples examined, age and sex distribution of patients, monthly distribution of isolates and distribution of isolates according to the species were investigated. The possible influence of environmental factors (the average monthly temperature and total monthly precipitation) on the incidence of campylobacteriosis was examined.

Materials and Methods

This was a study of sporadic, domestically acquired *Campylobacter* spp. enteritis during 5 year period, January 2003 till December 2007. The data were obtained from the Laboratory for Diagnostics of Enteric Infections of the Teaching Institute of Public Health of the County Primorsko-goranska (TIPH CPG) for the years 2003–2007 and were analyzed retrospectively. All stool and other samples from outpatients are processed in the TIPH microbiological laboratories, in the County Primorsko-goranska as well as in all other Croatian Counties. This makes the results between different Croatian Counties comparable.

Stool samples

Stools were obtained from outpatients with ADD and examined for presence of campylobacters. In the year 2003 stool samples were checked for the presence of campylobacters only when it was specified from the family doctor. From the year 2004 on every admitted stool was examined for campylobacters. Based on good laboratory evidence duplicates were identified and excluded from the study, only one isolate *per* patient within one month was counted and defined as a case. Samples were inoculated onto CCD agar (Casein hydrolisate, Cefoperazone, Desoxycholate), Bolton's modification (Biolife, Milan, Italy) and incubated at 42° C microaerobically in anaerobic jars (CampyPak, Becton Dickinson, Maryland, USA) for 48 hours. Suspected colonies from primary culture were subcultivated on blood agar plates with 5% sheep blood under the same conditions. Determination of the genus *Campylobacter* was made according to the characteristic macroscopic colonial and microscopical appearance, positive oxidase and catalase tests. Species was determined using hippurate test (positive for *C. jejuni*) and confirmed with API Campy system (bio Mérieux, Marcy-l'Étoile, France).

Demographical data

Demographical data were collected from databases released by Republic of Croatia – Central Bureau of Statistics, Census 2001. According to the Croatian National Institute of Public Health these official data are recommended to be used in all epidemiological studies until new population census. Based on these data County Primorsko-goranska (3,582 m^2, 6.3% of the total national territory) has 305,505 inhabitants (6.9% Croatian total)[14].

Environmental data

Meteorological data, temperatures (degrees Celsius, averaged *per* month) and rainfalls (liters *per* square meter) for the investigated time period, were obtained from the Meteorological and Hydrological Service, Republic of Croatia[15].

Statistical analysis

Statistical analysis of data was performed using Statistica for Windows, release 8.1 (StatSoft, Inc., Tulsa, OK, USA). Overall incidence throughout the investigated period and overall incidence by gender and by age were presented as the mean ± standard deviation (SD). One-way analysis for variance (one-way ANOVA) was used to test the differences between the number of specimens examined for *Campylobacter* spp. as well as between the numbers of cases according to the years. Tukey's test was used for post-hoc analyses. The differences in proportions of cases of C. *coli* according to years were compared using t-test for proportions. The differences between incidence rates, age groups and sexes were analyzed using Pearson χ^2-test. Multiple regressions analyses were done to test the relationship between several independent or predictor variables such as the average monthly temperature, total monthly precipita-

tion and month of the year and a dependent or criterion variable such as the number of isolates. Multiple linear regressions analyses were done for each year. All statistical values were considered significant at the p level of 0.05.

Results

During five years period, from January 2003 to December 2007, in the Laboratory for Diagnostics of Enteric Infections TIPH CPG, 30,164 stool samples were obtained from patients with ADD were tested for the presence of campylobacters (Table 1). The number of specimen tested for campylobacters in the population varied from 4,614 stool samples in 2003 to 7,105 in 2007. Each year the number was significantly higher than the preceding year ($p<0.001$). The isolation rate (overall number of stools positive for *Campylobacter* spp. divided by the number of stools analyzed) differed from 4.81% in 2003 to 7.89% in 2007. However, when the duplicates were excluded, the number of cases (primoisolates) ranged between 138 (2.99%) in 2003 and 275 (4.72%) in 2004 ($p=0.002$). During the observed period *C. jejuni* was the most frequent species and 1,093 (88%) of 1,242 primoisolates were identified as *C. jejuni* and 149 (12%) as *C. coli*. No other species was isolated. In the year 2003 and 2006 *C. coli* accounted for over 20% (24.6% and 20.9%) of cases, what was about four times more than in the year 2005 when only in 5.7% cases *C. coli* was identified (2003. *vs*. 2005. $p<0.001$; 2006. *vs*. 2005. $p<0.001$).

The overall annual campylobacter incidence rate in County Primorsko-goranska between 2003 and 2007 was 81.3±21.9/100,000. The incidence rate varied from 45.2/100,000 in 2003, to 101.5/100,000 in 2007 (Table 2). Sex and age distribution is also demonstrated in Table 2. Throughout the observed five year period male consistently experienced higher rates but the differences were not significant ($p=0.198$). Campylobacter incidence rates were highest in children under five years of age (484.4±129.1/100,000; $p<0.01$) and in those between five and nine years (226.5±60.5/100,000; $p<0.05$) in comparison to all other age groups. In the 20–29 years age group the incidence was high (100.8±27.8) but the difference compared to all other groups was not significant ($p>0.05$). The incidence was lower within older groups. The similar age and gender distribution patterns were observed consistently throughout all five years with minor annual variations.

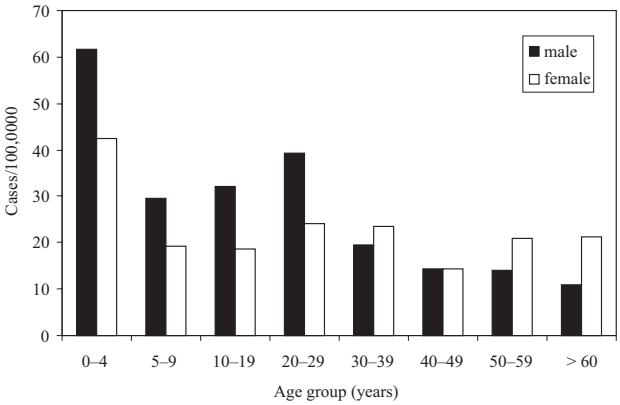

Fig. 1. Overall sex distribution of Campylobacter spp. incidence rates (cases per 100,000) in the County Primorsko-goranska (Croatia), 2003–2007.

Gender distribution according to the age groups is presented in Figure 1. Incidence in males was higher than in females from birth until late twentieth ($p<0.001$). Later on the differences between genders were less expressed and not significant.

The average monthly temperature and total precipitations were included in the study and are presented in Figure 2. Multiple regression analyses were done for each year for all the variables and the results were inconsistent, depending on the investigated year. Monthly average temperatures were significantly associated with campylobacter rates ($p<0.05$). Overall contribution of

TABLE 1
CAMPYLOBACTER SPP. CASES AND ISOLATION RATES DURING THE FIVE YEAR PERIOD (2003–2007) IN THE COUNTY PRIMORSKO-GORANSKA (CROATIA)

Year	Specimen examined for *Campylobacter* spp.	Isolation rate*	Cases**		*C. jejuni*/*C. coli***	
		(%)	N	%	N	%
2003		4.81	138	2.99	104/34	75.4/24.6
2004		7.64	275	4.72	257/18	93.5/6.5
2005		7.35	281	4.53	265/16	94.3/5.7†
2006		6.34	238	3.71	188/50	79.1/20.9
2007		7.89	310	4.36	279/31	90.0/10.0
Total	30,164	6.93	1,242	4.12	1,093/149	88.0/12.0

* The overall number of stools positive for *Campylobacter* spp. divided by the number of stools analyzed
** Only primoisolates
*** Annual *C. jejuni*/*C. coli* ratio
† In the year 2003 and 2006 *C. coli* accounted significantly more than in the year 2005 (2003 *vs*. 2005 $p<0.001$; 2006 *vs*. 2005 $p<0.001$; t-test for proportions)

TABLE 2
ANNUAL CAMPYLOBACTER INCIDENCE BY GENDER AND BY AGE IN THE COUNTY PRIMORSKO-GORANSKA (CROATIA)

	Year					
	2003	2004	2005	2006	2007	Mean±SD
Overall incidence*	45.2	90.0	91.9	77.9	101.5	81.3±21.9
Incidence* by gender						
Male	45.5	117.5	106.6	91.7	112.8	94.8±29.2
Female	44.9	64.4	78.3	65.7	90.9	68.8±17.2
						p=0.198**
Incidence* by age (years)						
0–4	334.6	549.7	454.1	414.2	669.2	484.4±129.1
5–9	124.2	241.1	284.9	248.4	233.8	226.5±60.5
10–19	35.7	82.3	115.2	76.8	115.2	85.0±32.9
20–29	55.7	106.6	128.4	96.9	116.2	100.8±27.8
30–39	31.9	78.5	73.6	61.4	76.1	64.3±19.3
40–49	17.9	53.8	31.9	27.9	37.9	33.9±13.3
50–59	27.3	54.5	54.5	54.5	66.9	57.6±6.2
60	14.6	26.3	32.1	32.1	39.4	34.5±4.2
						p<0.001***

*Cases *per* 100,000 person
**The differences between genders were tested using χ^2-test
****Campylobacter* incidence rates were significantly higher in children 0–4 years (all $p<0.01$) and 5–9 years (all $p<0.05$) in comparison to all other age groups (χ^2-test)

temperature was found significant for the year 2003, 2005 and 2007 ($p<0.05$) and the portion of temperature contribution was mostly expressed in 2003 (44.6%), 2005 (49.9%) and 2007 (49.5%) as it is shown in Table 3. Precipitation and month of the year were not associated significantly with number of cases ($p>0.05$). In Figure 2 all the analyzed data are presented graphically. It can be noticed that *Campylobacter* spp. cases were mostly detected in the warmer period of the year, from May till October with the peak occurring in June or July and then decreasing. A low detection rate in winter (January and February, even till April) was demonstrated. However, this pattern was not consistently expressed throughout each observed year. The most expressed difference was seen in 2004 when low number of cases was recorded during summer months (June, July and August) and in autumn (September to December) the frequency of cases was persistently high. In the year 2006 two marked peaks, in January and September could be noticed, with the lowest incidence in early spring (February to May) and December.

TABLE 3
CONTRIBUTION OF AMBIENT TEMPERATURE ON NUMBER OF CAMPYLOBACTER CASES BY YEAR (2003–2007)*

Year	β	s_β	*P*	*r*	Portion of contribution (%)
2003	0.832	0.357	0.048	0.536	44.6
2004	–0.180	0.328	0.598	0.076	1.3
2005	0.672	0.224	0.017	0.743	49.9
2006	0.371	0.441	0.424	0.138	5.2
2007	0.716	0.224	0.013	0.691	49.5

*Multiple regression analysis
β – coefficient of regression, s_β – standard error of coefficient of regression, r – coefficient of correlation

TABLE 4
CAMPYLOBACTER INCIDENCE RATES IN SOME EUROPEAN COUNTRIES AND THE COUNTY PRIMORSKO-GORANSKA (CROATIA)

Country	Incidence*	Reference
County Primorsko-goranska (Croatia)	81.3	our study
Finland	19.6–72.8	5
Scotland	60–160	12
England/Wales	78.4–103.7	16, 23
Switzerland	76.5	16
Sweden	73.9	16
Norway	39.5	16
The Netherlands	21.5	16

*Cases *per* 100,000 person

Discussion

During the observed period, number of domestically acquired cases of human campylobacteriosis in the County increased. The most prominent increase was noticed between 2003 and 2004 when new protocol was introduced in the stool processing and all stool samples admitted to the Laboratory were examined on the presence of campylobacters. In the same time period the incidence of salmonellosis was decreasing from 158.8/100,000 in 2003 to 76.6/100,000 in 2007 (data not shown) like in the European Union (EU) member countries[8]. According to available data, the average incidence of human campylobacteriosis show marked differences among the countries in Europe and in the world[11,16]. Evenmore, it has been registered that the annual incidence can show huge variations between regions in the same country[5,12]. The results of first European surveys conducted by ECDC (European Centre for Disease Prevention and Control) in 1998 and 1999 in 15 EU (European Union) countries showed that the notifications of campylobacteriosis *per* 100,000 inhabitants varied from 2.9/100,000 to 166.8/100,000 between countries, indicating a rising trend[17]. The incidence rate of *Campylobacter* infections among the same 15 reporting EU countries in the third quarter of 2007 was 6.9 *per* 100,000 persons, but this last study had limitations by its inability to compare data with prior reports due to a lack of consistency in reporting countries and systems[18]. Those differences clearly indicate discrepancy among European countries included in different studies, possibly due to diagnostic methods and procedures used in primary laboratories that investigate patient samples. Therefore, our results, similar to some of those mentioned, fit into the present situation in Europe (Table 4). Our study showed that campylobacters are important enteric pathogens in our region. Since this is the first epidemiological study showing that human campylobacteriosis represents an important public health problem in the County Primorsko-goranska, our results could be possibly extrapolated to other Counties and the whole Croatia.

As shown in our study, during the whole investigated period of five years, the vast majority among *Campylobacter* isolates belonged to *C. jejuni*. Our investigations showed that in the County Primorsko-goranska *C. jejuni* represented 88% of the isolates. That was expected because most of the population lives in the cities (237,649 or 77.8%) and almost half of that (144,043 or 47.1%) in the largest one, Rijeka[14]. Numerous authors confirm that *C. jejuni* and *C. coli* are the two of *Campylobacter* species most frequently isolated and that *C. jejuni* has been isolated more often than all the other species[2,11,16,17]. From the study carried out in 1985 in Zagreb area the difference in *Campylobacter* incidence between urban and rural populations was seen (71/100,000 to 99/100,000 respectively) with *C. coli* predominant in rural area[19]. Ten years later another study conducted in the same area showed a significant increase in the number of *C. jejuni* compared to *C. coli* infections, but the study from the neighboring regions (Bosnia and Herzegovina, Slovenia) still reported high incidence of *C. coli* among human and food isolates[20,21].

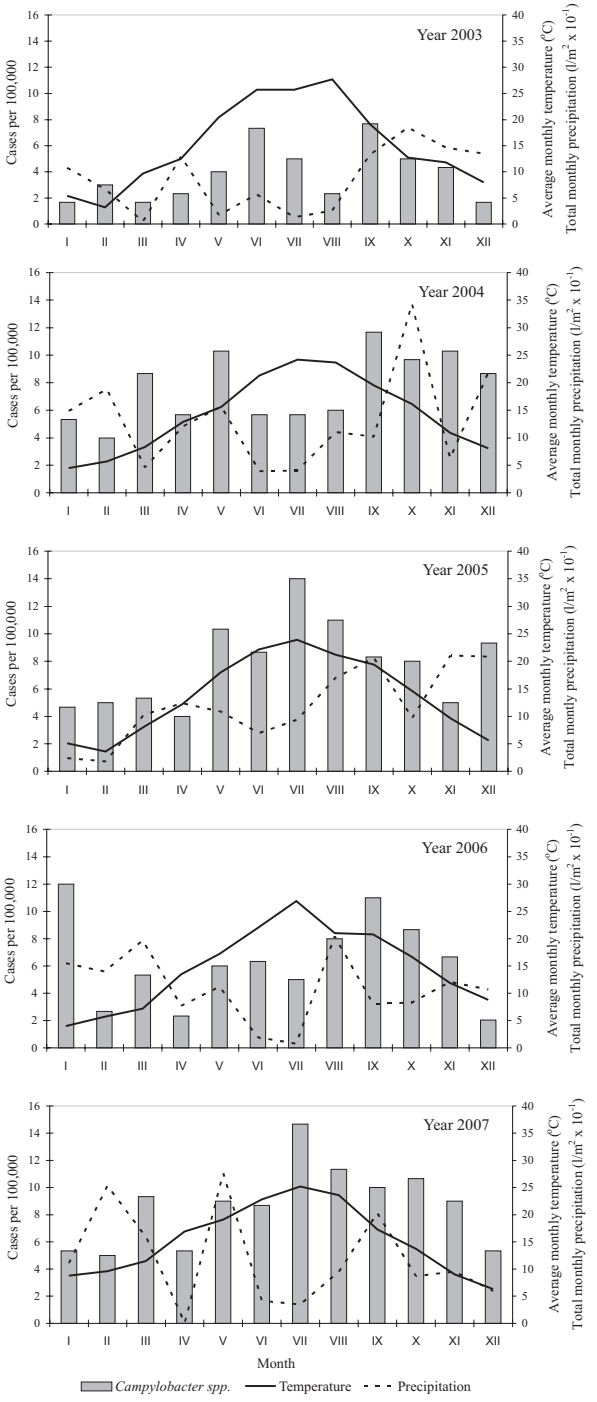

Fig. 2. The monthly distribution of incidence rate of laboratory confirmed human campylobacteriosis, the average ambient temperature and total precipitation observed in the County Primorsko-goranska (Croatia), 2003–2007.

Our findings about gender and age distribution, with the highest incidence of domestic *Campylobacter* infec-

tions found among young male patients, from birth till late twentieth, and children under the age of ten and in young adults (20–29 years old), match the pattern of campylobacter infections described in developed countries[11,22]. Higher male incidence is seen in almost every published study but has not been fully elucidated till nowadays[1,18,23]. This age pattern also indicates the necessity of education of young people, especially parents, mostly mothers with young children, considering the possible underestimated and insufficiently investigated interhuman transmission of campylobacters[24]. Possible feco-oral person to person spread could be mostly relevant among young children who do not practice good hygienic habits[25].

We also examined the correlation of temperature and precipitation with the incidence of human campylobacteriosis in our County. A lot of studies show a distinct seasonality in campylobacter transmission to humans but the timing of the peak differs among countries. Comparison of these results is difficult due to the different methodology used in the studies, but they clearly show that the peak has been consistently repeating in the same month or even week of the year[9,10,12,13,23,26]. In some countries a peak in spring is shown and in others summer peak is noticed[8]. Data from Denmark pointed that the maximum temperatures preceded the incidence of human campylobacteriosis up to 4 weeks[27], but also a delay as long as 3–4 months in the effect of temperature on the number of the campylobacter cases is described in some countries[9,28]. Our investigations showed no distinct seasonality but the increase in campylobacter incidence was predominantly associated with increased ambient temperature. Considering the characteristics of Mediterranean climate in our County high incidence of campylobacteriosis was observed mainly from May forward. On the contrary, the amount of precipitation, what could influence on bacterial survival in the environment, was not significantly associated with the number of campylobacter cases. Kovats RS et al. also suggest that climate can contribute to campylobacter transmission, but that it was not the main driver of observed seasonality[9]. The food-borne route of infection also can not completely explain the seasonal peak of campylobacteriosis, because agricultural activities, recreational contact, bird activities, could also represent potential transmission routes. The possible role of flies as mechanical or biological vectors in the transmission of campylobacters needs to be further elucidated[29,30]. Changes in human eating habits and behavior according to the season and weather conditions must be also taken into account.

The observed increases in the incidence and isolation rate emphasize the importance of this pathogen as a cause of diarrhea in the County Primorsko-goranska (Croatia). This increase in the number of cases could be the result of greater awareness of this bacterial disease potential and the subsequent increase in the number of stool samples examined for *Campylobacter* spp. This study pointed out some unknown data about human outpatient campylobacteriosis in our County: age and sex distribution, the incidence of *C. coli* and the influence of environmental factors. It would be also important to perform a long term study which could enable the comparison with forthcoming results regarding the new law regulations and mandatory notification of human campylobacteriosis on the national level.

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EPIDEMIOLOŠKE OSOBITOSTI HUMANE KAMPILOBAKTERIOZE U ŽUPANIJI PRIMORSKO-GORANSKOJ (HRVATSKA), 2003–2007

S A Ž E T A K

Cilj rada bio je utvrditi incidenciju kampilobakterioze u Primorsko-goranskoj županiji (Hrvatska) u razdoblju od 2003. do 2007. godine te istražiti moguću povezanost s okolišnim čimbenicima (prosječnom mjesečnom temperaturom i ukupnom mjesečnom količinom padalina). Retrospektivno su analizirani podaci (broj pretraženih uzoraka stolice, dob i spol bolesnika, distribucija izolata po mjesecima i izoliranim vrstama kampilobaktera) laboratorija za dijagnostiku crijevnih infekcija Nastavnog zavoda za javno zdravstvo Primorsko-goranske županije. Tijekom proučavanog razdoblja 30,164 uzorka stolice bili su pretraženi na kampilobaktere. Bakterije roda *Campylobacter* identificirane su u 1,242 (4,12%) uzorka. Ukupna incidencija na godišnjoj razini iznosila je 81,3±21,9/100,000 stanovnika. *Campylobacter jejuni* izoliran je u 1,093 (88%), a *C. coli* u 149 (12%) bolesnika. Istraživanje je pokazalo dobnu raspodjelu bolesnika karakterističnu za industrijski razvijene zemlje. Bolesnici su bili uglavnom djeca ispod 5 godina starosti (484,4±129,1/100,000, $p<0,001$) te između 5 i 9 godina (226,5±60,5/100,000, $p<0,05$). Cijelo ispitivano razdoblje zastupljeniji su bili bolesnici muškog spola, a značajno ih je bilo više u nižim dobnim skupinama, od rođenja, do kasnih dvadesetih godina ($p<0,001$). Također su uočene mjesečne varijacije u broju izolata unutar godine. Učestalost izolacija kampilobaktera bila je značajno povezana s prosječnim mjesečnim temperaturama ($p<0,05$), ali ne i sa količinom padalina. Neophodno je istražiti incidenciju kampilobakterioze za cijelu državu. Također je nužno detaljnije istražiti uzroke uočenih razlika među mjesecima unutar godine, izvora infekcije i povezanost s načinima prijenosa infekcije u ljudi.