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Campylobacter jejuni and *Campylobacter coli* diarrhoea in rural and urban populations in Yugoslavia

T. POPOVIĆ-UROIĆ

Department of Clinical Microbiology, University Hospital of Infectious Diseases, Dr Fran Mihaljević, Zagreb 41000, Mirogojska 8, Yugoslavia

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SUMMARY

During a 4-month period during the summer of 1985, campylobacters were isolated from 338 (16·3%) of 2080 patients with acute diarrhoea attending the University Hospital of Infectious Diseases in Zagreb. Of these isolates 220 (64·1%) were *Campylobacter jejuni* and 118 (34·9%) were *C. coli*. The patients were drawn from three residential zones in and around Zagreb: inner city, peripheral city and rural. Incidences of campylobacter diarrhoea ranged from 71 per 100000 per year in inner city residents to 99 per 100000 per year in the rural residents. Most infections were in young children; the incidence in infants ranged from 800 to 2500 per 100000 per year in the inner city and rural zones respectively. The isolation rate from faecal specimens of infants from the rural zone was 61%. The ratio of isolation rates in males and females (all ages) was 1·1:1, but in infants it was 0·7:1 and in patients over the age of 65 years it was 0·4:1. The incidence of *C. coli* in the rural zone was four times that in the inner city and twice that in the peripheral zone.

This survey shows that campylobacter infection in Zagreb has distinctive epidemiological features. The transmission of infection appears to be midway between that found in industrialized and developing countries, and there is an unexplained excess of *C. coli* infection.

INTRODUCTION

Campylobacters are now recognized as one of the most important causes of diarrhoea in human beings in both developing and developed countries (Georges-Courbot *et al.* 1986; Johnson *et al.* 1985). Yugoslavia is no exception. At the University Hospital of Infectious Diseases in Zagreb over 20000 faecal specimens are examined each year of which about 800 yield campylobacters. By comparison, a similar number of shigellas and about 1000 salmonellas are isolated annually. This high turnover, which represents an estimated one half of all patients sampled for acute diarrhoea in and around Zagreb, provided an opportunity to carry out a demographic survey to find out which groups were most affected by campylobacter enteritis and at what intensity. Additional information was also sought on *Campylobacter coli* infections, as Zagreb has the highest recorded ratio of *C. coli* to *C. jejuni* infection on record (Kalenić *et al.* 1985).

Table 1. *Population, notifications of infective diarrhoea, isolation rates and incidence of campylobacter infections in Zagreb, June–September 1985*

Zone of residence	Population served	Notified diarrhoeas Zagreb*	Specimens examined at University Hospital (% of notified diarrhoeas)	No. positive		Incidence (per 10000/year)		
				C. jejuni	*C. coli*	*C. jejuni*	*C. coli*	Total
Inner city area	167619	522	282 (54)	33	7†	58·8	12·6	71·4
Peripheral city area	677783	3037	1305 (43)	135	58†	59·8	25·6	85·4
Rural area	317248	956	493 (52)	52	53†	48·9	50·2	99·3
Total	1162650	4515	2080 (46)	220	118	56·7	30·6	87·2

* Notifications of 'infective diarrhoea' to Republican Public Health Institute.
† $P < 0{\cdot}01$ for differences in *C. coli* isolation rates between zones.

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METHODS

Population

The University Hospital of Infectious Diseases serves the city of Zagreb and the surrounding region which together have a population of 1·2 million. The population divides naturally into three residential zones:

(1) Inner city: a central urban zone where most people live in flats;

(2) Peripheral city: an industrial zone where the inhabitants live in small family houses as well as flats;

(3) Rural: a farming and agricultural zone where people live mostly in their own farm houses where they raise their own domestic animals and work on the land. The populations of each of these zones are shown in Table 1.

Sampling patterns

The University Hospital runs an open access clinic, 24 h of the day, to which anyone can attend without referral. Most other hospitals in Zagreb require doctors notes or have limited hours of access. Because of this open access, patients are drawn from all three zones in roughly equal proportions. Table 1 shows the numbers of patients seen at the University Hospital relative to the numbers of patients with acute diarrhoea notified in the Zagreb area (in Yugoslavia 'infective' diarrhoea is statutarily notifiable and the estimated compliance is 75%). Proportionally fewer children under the age of 4 years from the inner city and peripheral city zones were seen at the University Hospital relative to the rural zone, as city children traditionally attend local paediatric services which are well developed in the city.

Collection of data

The age, sex and zone of residence of all patients presenting to the University Hospital with acute diarrhoea during the 4 months from 1 June to 30 September 1985 were recorded. Faecal specimens (occasionally rectal swabs) from all the patients were examined and the presence of campylobacters recorded.

Differences of probabilities were tested using chi-square analysis.

Laboratory methods

Faecal specimens were examined in the Department of Clinical Microbiology in the University Hospital, usually within 2 h of collection but occasionally stored overnight at 4 °C. Each faecal specimen was plated on Preston medium (Bolton & Robertson, 1982) and on Butzler's Virion medium (Goossens, De Boeck & Butzler, 1983). The plates were incubated at 42 °C in an atmosphere with the oxygen lowered by a method based on the Fortner principle similar to that described by Karmali & Fleming (1979). After incubation for 48 h campylobacters were identified by colony morphology, Gram stain, positive catalase and oxydase reactions, susceptibility to nalidixic acid (30 μg disc) and resistance to cephalothin (30 μg disc). The distinction between *C. coli* and *C. jejuni* was determined by hippurate hydrolysis performed as described by Harvey (1980). Local reference strains of *C. jejuni* (581/83) and *C. coli* (582/83) were used as controls; the identity and reactions of these strains were confirmed by Dr M. B. Skirrow at the Worcester Royal Infirmary Laboratory in the United Kingdom.

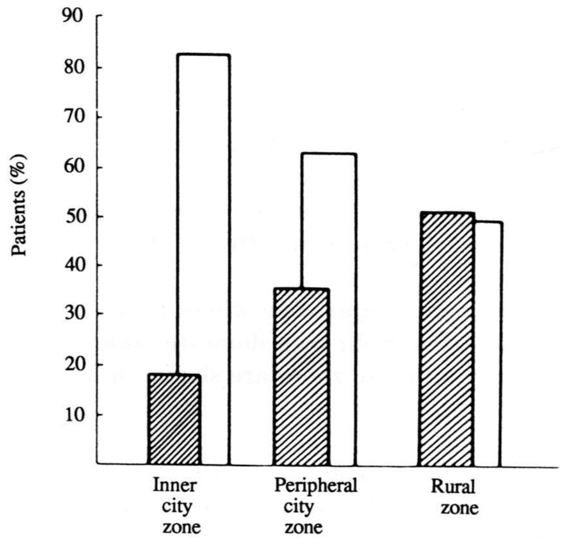

Fig. 1. Relative proportions of *C. coli* and *C. jejuni* diarrhoea in each zone of residence. □, *C. jejuni*; ▨, *C. coli*.

RESULTS

The total number of patients sampled during the 4-month period of the study was 2080; 29% were admitted to the Hospital. Campylobacters were isolated as sole presumptive pathogen from 338 (16·3%) specimens and from an additional 5 specimens with salmonellas or shigellas, giving a total of 343 (16·5%). Only patients who had campylobacter infections alone are included in the following analysis.

Sampling rates in all zones were similar, except that the rate for infants in the inner city zone was only half the rate of the other two zones, and that for the age group 5–14 years was twice the rates for the other two zones. Over the age of 15 years the rates steadily decreased in all three zones of residence.

Distribution of infection by residential zone

The campylobacter isolation rates and incidences in the three residential zones are shown in Table 1. There was a progressive increase in incidence from 71 per 100000 per year in the inner city zone to 99 per 100000 per year in the rural zone. The excess incidence in the rural zone was due to *C. coli*, which was isolated four times as often than from the inner city zone patients (Table 1). *C. coli* accounted for just over half of the campylobacter infections in the rural zone (Fig. 1).

Distribution of infection by age

Crude figures show that most isolations were obtained from children (Fig. 2). This was most pronounced in the rural zone where 66·6% of all patients with campylobacter diarrhoea were aged less than 5 years, compared with 50·8% and 40·0% for the peripheral and inner city zones respectively ($P = < 0{\cdot}01$ between figures for each zone). When these figures are expressed as incidences the excess of infections in children is accentuated, particularly in infants less than 1 year old

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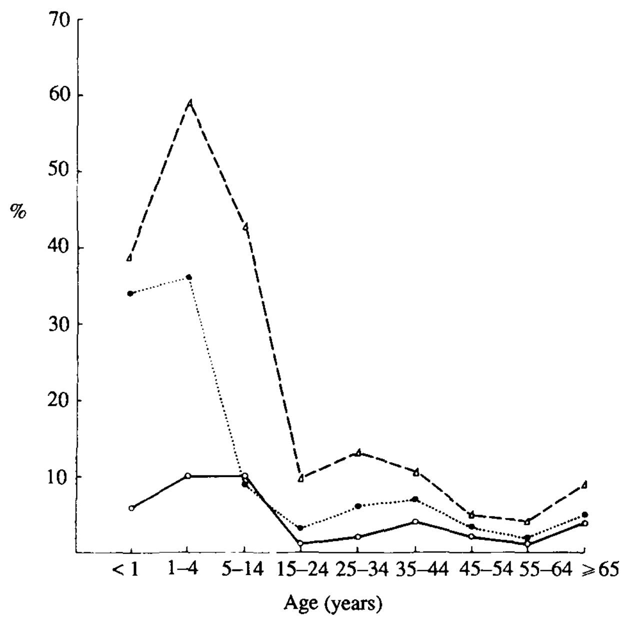

Fig. 2. Patients with campylobacter diarrhoea by age and zone of residence. ○—○, inner city zone ($n = 40$); △---△, peripheral city zone ($n = 193$); ●····●, rural zone ($n = 105$).

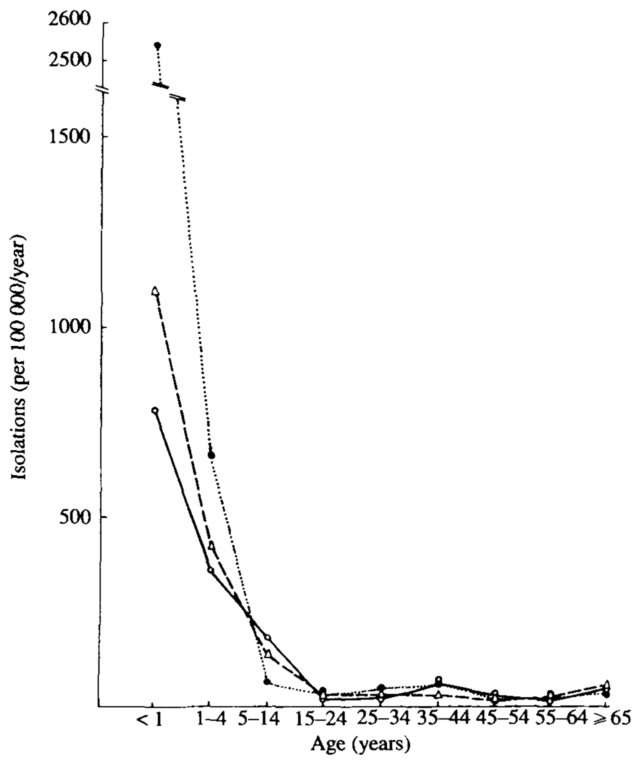

Fig. 3. Age specific incidences of campylobacter diarrhoea by zone of residence. Symbols and numbers of patients as for Fig. 2.

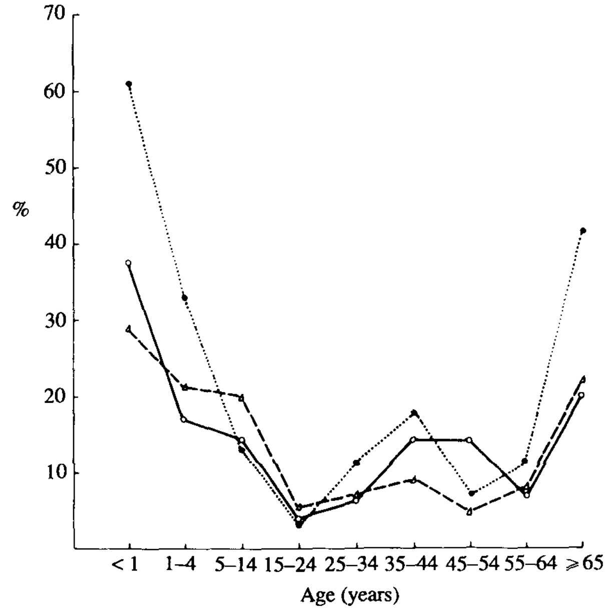

Fig. 4. Percentage faecal specimens positive for campylobacters by age and zone of residence. Symbols and numbers of patients as for Fig. 2.

(Fig. 3). This was most striking in the rural zone where rate of 2550 per 100000 per year was recorded in this age group, four times the figure for the inner city zone. High infant infection rates were followed by sharp falls to low rates in children over the age of 5 years. The crossover of all three plots representing the three different zones of residence occurred before the age group 5–14 years. Infection rates in patients aged 15 years or more remained more or less constant.

Percentage of faecal specimens positive by age

The percentage of faecal specimens positive within each group in the three zones of residence are shown in Fig. 4. In general, the highest figures were again in children less than 1 year old in whom 38% of faecal specimens were positive. This percentage was even higher (61%) in infants from the rural zone. High isolation rates were also evident in patients aged 65 years or more and there was a modest peak (11·7%) in patients aged 35–44 years.

Distribution of infection by sex

The combined male/female (M/F) ratio for the incidence of campylobacter diarrhoea was 2·3:1. Equivalent M/F ratios for the three residential zones were inner city 1·1:1, peripheral city 2·7:1 and rural 2·5:1. The M/F ratios of isolation rates for different age groups (three zones combined) showed an excess of females in infants and in the patients over the age of 65 years (M/F = 0·7:1 and 0·4:1 respectively). The M/F ratio for *C. coli* infections was 1·7:1 and for *C. jejuni* infections 2·4:1.

DISCUSSION

The figure of 87 per 100000 for the crude annual incidence of campylobacter diarrhoea is subject to two biases which partly cancel each other out. First, the figure relates to summer months when the incidence is higher than in other times of the year; the average annual rate in 1985 was 56 per 100000. Second, not all specimens from patients with diarrhoea would have been tested at the University Hospital laboratory, although those that were tested represented 46% of notified cases of 'infective' diarrhoea (Table 1). Thus the true figure is probably near to the original 87 per 100000 per year. This is at the upper end of the range of incidences reported or derived from reports from North America (33–97 per 100000 per year: Dilworth, 1983; Coles *et al* 1985; Thompson, Cahoon & Hodge, 1986) or from England (58 per 100000 per year: Skirrow, 1987). The incidence in the rural zone population (99 per 100000 per year) exceeded these rates. High incidences in rural relative to urban areas have been observed previously (Kist & Rossner, 1985; Sibbald & Sharp, 1985) and in farm residents (Thompson, Cahoon & Hodge, 1986).

Although these rates are not exceptionally high, they contain striking differences in the age distribution of infection. Most surveys in developed countries show that around 20% of all infections are in children below the age of 5 years (45% in the survey reported by Dilworth, 1983), whereas in the present study the proportion was 54% rising to 67% in the rural population. These differences are even more striking in infants aged less than 1 year, in whom the rates of infection ranged from 787 per 100000 per year in the inner city to 2550 per 100000 per year in the rural populations (Fig. 3). These figures are 5–15 times the sort of rates reported in industrialized countries (Sibbald & Sharp, 1985; Thompson, Cahoon & Hodge, 1986; Skirrow, 1987) but are not as high as those found in developing countries, e.g. 40% per year (Calva *et al.* 1988). Moreover, unlike reports from developing countries, there were few double infections.

Incidence values are prone to distortions from irregularities of sampling; for example infants are more likely to be sampled than older people because they are more vulnerable. In the present study the sampling rate of infants in the inner city zone was only half that in the other zones for reasons given previously. Sampling bias is largely eliminated by analysing the percentage of faecal specimens positive within each age group; it is another way of looking at the data and it is often illuminating. A comparison of such results from Zagreb (Fig. 4) with those of Skirrow (1987) serve to emphasize the high rates of infection in Zagreb infants relative to those in England. The plots in Fig. 4 are almost a mirror image of those shown in Fig. 3(*b*) in Skirrows paper and there is a 30-fold difference in isolation rates in children less than 1 year of age.

The male/female (M/F) ratio of 2·3:1 for infection was considerably higher than that of around 1·3:1 commonly reported. This was almost certainly due to a sampling bias, for twice as many males as females were sampled (2·1:1) yet the M/F ratio for diarrhoea notifications was 1:1 and that for percent faecal samples positive was also: (M, 16·4%; F, 15·9%). There is no obvious explanation for the excess isolation rates in female infants and women over the age of 65 years.

An unexpected finding was the striking difference between the isolation rates of

C. coli in the three residential zones. The *C. coli* isolation rate in rural residents was four times that in inner city residents and it accounted for the excess incidence of campylobacter enteritis in rural residents; the isolation rates of *C. jejuni* in the three zones were not significantly different (Table 1). According to a previous survey the proportion of *C. coli* isolation would have been even higher had the survey been carried out in the autumn (Kalenić *et al.* 1985). As *C. coli* is particularly associated with pigs (Banffer, 1985; Bolton, Dawkins & Hutchinson, 1985), the local tradition of home slaughter and processing of pigs at the end of the summer is a possible explanation for this, although Kalenić *et al.* (1985) observed that *C. coli* was also predominant (57 % of strains) in Zagreb poultry. The latter is an unusual finding paralleled only in a survey in France (Marinescu *et al.* 1987).

Preliminary enquiries showed that twice as many campylobacter infected patients from the rural zone as from the city zones regularly ate home processed meats, drank raw milk, or lived in households with pigs, poultry or cattle – all known risk factors for acquiring campylobacter infection (Blaser, Taylor & Feldman, 1983). Conversely, twice as many city dwellers lived in households with pipe-borne town water and flushing sewage disposal system. These were uncontrolled enquiries, but the results serve as pointer for future case control studies. There was no significant difference between the proportions of patients infected with *C. jejuni* or *C. coli* in relation to these factors (data not shown).

In summary, this survey shows that campylobacter enteritis in Zagreb has distinctive epidemiological features. The incidence is high, with a large proportion of infections in children less than 1 year of age, particularly in the rural area surrounding the city. The pattern is intermediate between that of industrialized countries and developing countries. There is an unexplained predominance of *C. coli* infections in the rural population. A case control study designed to define and quantify possible risk activities, in particular the home processing of pork and other meats, would be a logical way forward.

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