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Campylobacter jejuni isolations from Mexican and Swedish patients, with repeated symptomatic and/or asymptomatic diarrhoea episodes

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SUMMARY

The presence of different *Campylobacter jejuni* serotypes in Swedish patients with diarrhoea and in Mexican patients with or without diarrhoea was investigated with special reference to repeated isolations during the course of infection and to symptomatic and asymptomatic episodes. The study included 136 *C. jejuni* isolates from 62 Mexican children and 173 isolates from 68 Swedish patients. The bacteria were serotyped for heat-stable (HS) and heat-labile (HL) antigen.

Swedish patients, all with symptoms, were in general only infected with one serotype and were rarely reinfected. Mexican patients on the other hand were in general infected with mixed serotypes and frequently reinfected without symptoms with new, different serotypes, a finding which is in concordance with a theory of an induced immunity to surface antigens.

INTRODUCTION

Campylobacter jejuni is an important case of acute diarrhoeal disease occurring in both sporadic and epidemic form (Blaser *et al.* 1983; Svedhem & Kaijser, 1980; Skirrow, 1987; Jones *et al.* 1981). Repeated enteric infection with this organism is particularly prevalent in children in developing countries where most of the cases occurring in children over 2 years old are asymptomatic (Glass *et al.* 1983; Calva *et al.* 1988; Mathan *et al.* 1984; Georges-Courbot *et al.* 1986; Alabi *et al.* 1986). It has been claimed that different strains appear in the repeated infections, but that a limited number of serotypes dominate (Blaser *et al.* 1986; Karmali *et al.* 1983).

Several different factors possibly associated with virulence have been suggested for thermophilic campylobacter such as motility, invasiveness, adhesiveness, enterotoxin and/or cytotoxin production (Guerrant *et al.* 1987; Klipstein & Engert, 1984; Johnson & Lior, 1986; Newell & McBride, 1985; McSweegan & Walker, 1986). Klipstein *et al.* (1985) found a strong correlation between the clinical outcome in the infected host and certain properties of the infecting strain of *C. jejuni*. In our recent study from Sweden, we concluded that there was no

correlation between the nature and severity of clinical signs and the presence of a specific serotype (Kaijser & Sjögren, 1985).

The purpose of this investigation was to study the presence of different *C. jejuni* serotypes in patients in Sweden and Mexico with special reference to repeated isolations during the course of infection and also to compare symptomatic and asymptomatic episodes.

MATERIALS AND METHODS

Bacteria. The study included 136 *C. jejuni* isolates from 62 Mexican children and 173 isolates from 68 Swedish patients.

Stool specimens were collected by rectal swabs and were cultivated for *Campylobacter* sp. as described earlier (Sjögren *et al.* 1987; Calva *et al.* 1988). Briefly, samples from the Swedish patients were transported in MSM (modified Stuart medium) or SMTM medium (semisolid motility test medium) (Sjögren *et al.* 1987). The latter were also used as an enrichment medium. All samples were plated directly within 24 h of sampling and again after 24–48 h of enrichment. The culture media contained Colombia agar (BBL), horse blood and the selective antibiotics vancomycin, polymyxin B and trimethoprim. Identification of *Campylobacter* sp. was as described by Skirrow (1977). *C. jejuni* and *C. coli* were not differentiated. Local experience shows that 90% of the strains were *C. jejuni*.

The samples from the Mexican patients were examined by standard procedures with Campy-Bap medium (BBL) made with Brucella agar, lysed sheep erythrocytes and with the selective antibiotics vancomycin, trimethoprim, polymyxin B, amphotericin and cephalothin (Calva *et al.* 1988).

Strains were stored by lyophilization of a streak from the whole primary, swarming culture.

Serotyping. All serotyping of the Swedish as well as the Mexican strains was performed by the same person at the Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden. Upon subcultivation of the lyophilized strains, all morphologically different colonies were serotyped separately if possible. However many of the cultures appeared as morphologically homologous swarming growth.

Serotyping of the heat-stable HS antigen was performed according to the procedure described by Penner *et al.* (1983) using the indirect haemagglutination technique. The heat-labile HL antigen was determined by the method of Lior *et al.* (1982) using live bacterial cells suspended in buffered PBS-saline and adsorbed antisera to remove antibodies against homologous heat-stable and heterologous heat-labile antigens.

The hyperimmune antisera used for both groups were produced with reference strains from Drs Penner and Lior and were kindly supplied by the Culture Collection, University of Göteborg (CCUG). Twenty-three HS and 20 different antisera were used based on the typing results with the strains in our recent study (75–95%) (Kaijser & Sjögren, 1985) and experience from other epidemiological studies (Skirrow, 1987; Patton *et al.* 1985). To avoid rough strains or transient antigens, the isolates were subcultivated and retested five times before being recorded as a non-typable strain for the antisera used in the two systems.

Table 1. *Campylobacter isolates from Swedish patients with symptoms of infection*
(The patients illustrated in the table were selected because they had two or more specimens collected with at least one week intervals between.)

		Sampling period (weeks)				
Patients	Initial	1	2	3	4	5–8
1	2:4*	2:4	—	—	—	
2	NT:1**	—	—	16·9	—	—
3	6:9	—	—	—	6·9	—
4	15:2	—	—	—	—	15:2
5	3:2	—	3:2	—	3:2	—
6	2:4	—	—	—	2:4	—
7	2:4	—	—	—	—	2:4
8	2:4	—	2:4	—	—	—
9	8:7	8:7	—	—	—	—
10	6:4	6:4	—	—	—	—
11	4:1	4:1	—	—	—	—
12	1:1	1:1	—	—	—	—
13	5:2	5:2	—	—	—	—
14	2:4	2:4	—	—	—	—
15	2:4	2:4	—	2:4	—	—
16	NT:4	NT:4	—	NT:4	—	—
17	4:4	—	—	—	—	8:16
18	16:4	16:4	—	—	—	—
19	2:4	—	—	—	2:4	—
20	1:1	—	—	—	1:1	—
21	2:19	—	2:19	—	—	2:19
22	37:2	37:2	—	—	—	—
23	1:2	1:2	—	—	—	—
24	1:2	—	—	—	—	1:2
25	4:9	—	—	—	—	13:11
26	8:2	—	—	—	8:2	—
27	NT:2	—	—	NT:2	—	—
28	10:2	—	—	10:2	—	—
28	8:4	8:4	—	—	—	—

*, HS:HL (Heat stable:Heat labile) serotype.
**, NT, not typable strains using our antisera.

Patients. The Mexican children ($n = 62$), all under 4 years of age, were from selected families living in a poor urban community. Weekly stool specimens were obtained over the 12-month study period in 1985–6. The campylobacter infection was said to be asymptomatic when there were at least 5 consecutive symptom-free days before and after the isolation of campylobacter in faeces. A symptomatic episode for the Mexican group defined as a diarrhoeal episode occurring within 5 days, before or after, of the faecal isolation of campylobacter.

The Swedish strains were isolated over a period of one year (1985–6) from 68 patients all of whom were aged 15 or more and were seeking medical care for diarrhoea at the Department of Infectious Diseases, Østra Hospital, Göteborg, Sweden. Rectal swab specimens were collected weekly during the 2-month follow-up period after the initial diarrhoeal episode. Symptomatic infection for the Swedish patients was recorded when the patient reported that he had had

Table 2. *Campylobacter strains (n = 31) from Mexican children with symptoms, serotyped according to the heat-stable HS-antigen and the heat-labile HL-antigen*

Serotype (HS)**	No. of strains	Frequency (%)	Serotype (HL)***	No. of strains	Frequency (%)
37	4	12·9*	21	10	32·2
15	3	9·7	2	8	25·8
1	3	9·7	36	5	16·1
8	3	9·7	4	2	6·5
4	2	6·5	11	2	6·5
5	1	3·2	13	1	3·2
6	1	3·2	1	1	3·2
18	1	3·2	20	1	3·2
23	1	3·2	12	1	3·2
Total typable	19	61·3		31	100·0
Not typable	12	38·7		0	100·0
	31	100·0		31	100·0

* $P < 0{\cdot}05$ compared to strains from patients without symptoms. (Table 3).

** Hyperimmune antisera against reference strains with HS antigens 2, 3, 9, 10, 11, 13, 14, 16, 17, 19, 20, 21, 22 and 25 were also tested, but none of the strains belonged to any of these serotypes.

*** Hyperimmune antisera against reference strains with HL antigens 5, 6, 7, 8, 9, 10, 16, 17, 18, 19 and 22 were also tested, but none of the strains belonged to any of these serotypes.

diarrhoea and when campylobacter was isolated from the stool sample. Two or more specimens were collected during the acute phase of the episode within the first week. In 29 of the 68 patients the interval between the first specimens and the next ones was a week or longer (Table 1).

Statistical calculations. Differences of probabilities were tested by using the Chi-square test with the Yates' correction factor (Colton, 1974).

RESULTS

In the Swedish study, most of the patients were infected with one strain regardless of whether the patient was infected in Sweden or abroad. During the whole period of study the patient excreted the same serotype, indicating that no or few reinfections occurred. Table 1 presents the serotypes of the isolates from 29 of the 68 patients from whom samples were collected at intervals greater than one week.

In the Mexican children at the time of the campylobacter isolation, 31 child episodes were diarrhoea-associated (Table 2), while 105 episodes were asymptomatic (Table 3). During the follow-up period 38 (61·3%) of the patients had asymptomatic epidoses only. Six of the patients had both symptomatic and asymptomatic episodes; 1 child had 2 episodes, 1 had 4, 3 had 5 and 1 child had 11 episodes during 1 year. The children were repeatedly infected with new serotypes, and 26 out of the 62 patients (41·9%) were infected with mixed *Campylobacter* sp. at the time of isolation regardless of whether the episodes were symptomatic or asymptomatic. Table 4 illustrates the serotypes found in patients where three or more samples were typed during the 1-year study period.

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Table 3. *Campylobacter strains (n = 105) from Mexican children without symptoms, serotyped regarding the heat-stable-HS-antigen and the heat-labile-HL-antigen*

Serotype (HS)	No. of strains	Frequency (%)	Serotype (HL)**	No. of strains	Frequency (%)
13	8	7·6	21	26	24·8
21	8	7·6	2	17	16·2
5	7	6·7	9	17	16·2*
8	6	5·7	4	13	12·4
1	6	5·7	36	10	9·5
11	5	4·8	11	8	7·6
4	4	3·8	20	1	7·6
2	4	3·8	13	1	0·95
14	4	3·8	17	1	0·95
9	3	2·9	8	1	0·95
37	3	2·9	—	—	—
23	3	2·9	—	—	—
16	3	2·9	—	—	—
15	2	1·9	—	—	—
25	2	1·9	—	—	—
3	2	1·9	—	—	—
6	1	0·95	—	—	—
17	1	0·95	—	—	—
18	1	0·95	—	—	—
22	1	0·95	—	—	—
10	1	0·95	—	—	—
19	0	0·0	—	—	—
20	0	0·0	—	—	—
Total typable	75	71·4	—	95	90·5
Not typable	30	28·6		10	9·5

*$P < 0·05$ compared to strains from patients with symptoms (Table 2).

** Hyperimmune antisera against reference strains with HL antigens 5, 6, 7, 8, 9, 10, 16, 17, 18, 19 and 22 were also tested, but none of the strains belonged to any of these serotypes.

When the strains isolated from Mexican patients with symptoms were compared to those from patients with no symptoms, the same HS antigens and HL antigens could with few exceptions be identified in the two groups (Tables 2, 3). The HS antigen, serotype 37, was significantly ($P < 0·05$) more common in the symptomatic group (Table 2) while the HL antigen, serotype 9 ($P < 0·05$) was more common among the asymptomatic children (Table 3).

In the Swedish patients with symptoms serotypes 2 ($P < 0·05$), 1 and 4, were the most frequently identified, HS-antigens; in the Mexican patients with or without symptoms serotypes 1, 8, 5, 13, and 21 were the most common. The HL-antigens 4 ($P < 0·05$), 2 and 1 were most commonly found in the Swedish group and HL-antigens 21 ($P < 0·001$), 2 and 9 in the Mexican group.

DISCUSSION

The importance of *C. jejuni* as a cause of sporadic and epidemic outbreaks (Jones *et al.* 1981; Riley & Finch, 1985; Skirrow, 1987), as well as for travellers'

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Table 4. *Campylobacter isolates from Mexican children with or without symptoms of infection*
(The children illustrated in the table were selected because they had three or more specimens serotyped.)

Patient numbers	Initial samples	Sampling period (weeks) 1	2	3	4	5	6	7	8	11	13	14	20–30	31–40
1	NT:4*	·	·	·	·	21:21	NT:9	15:11	1:2	·	·	·	·	
							NT:4	NT:4						
	−					−	+	+	+					
2	NT**:4	·	5:NT	·	15:13	·	·	·	·	·	·	·	18:21	·
	−		−		−								−	·
3	5:11	·	·	·	4:9	·	·	·	·	·	·	23·4	·	·
												4:1		
	−				−							−		
4	15:36	·	·	·	·	5:NT	·	5:NT	·	·	·	4:36	4:36	·
												5:2		
	+					−		−						
5	13:9	16:3	·	NT:NT	·	·	·	·	·	·	·	·	NT:12	37:17
		13:9											NT:20	NT:20
	−	−		−									+	−
6	13:4	NT:11								1:NT				
	−	−								−				
7	21:21												NT:2	16:2
													21:21	NT:21
	−												−	−
8	6:21	NT:NT			23:19	4:9	21:11		5:9	NT:36	37:4	2:4	23:4	NT:2
	17:2				NT:9	13·4	9:21			9:21	13:4		14:9	9:21
	−	−			−	−	−		−	−	−	−	+	−
9	25:21												NT:4	NT:9
													NT:9	
	−												−	−
10	25:21		25:21	9:36			14:11							
	−		−	−			−							
11	5:9	21:11									NT:4			
	−	−									−			

12	4:9				1:2		8:21		21:20	
	–				NT:21		+		–	
					–					
13	11:2	1:36	13:2	8:4						14:21
			11:2							8:4
			8:4							
	–	–	–	–						–
14	1:21	16:2		NT:NT						
		1:21								
	–	–			–					
15	16:2				NT:21	NT:21				
	–				8:36	–				
					–					
16	9:2	NT:4			37:21	1:2				
	21:21	NT:21								
	–	+			+	+				
17	NT:21				NT:12					
					13:2					
					18:21					
	–				+					

*, HS:HL (Heat-stable:heat-labile) serotype.
– asymptomatic, + symptomatic.
**, not typable strains using our antisera.

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diarrhoea (Ryder *et al.* 1981; Speelman, 1983; Jertborn, 1988), is nowadays well established.

By serotyping strains originating from Mexican and Swedish patients, and identifying both the HS and the HL antigen, it was shown that both the frequency of colonization and the number of strains colonizing the patients in the two groups were different. Most of the Mexican children had three or more episodes during the study-year and one child was infected as many as 11 times with different campylobacter isolates during the study. Out of the 62 patients, 41·9% were also infected with mixed *Campylobacter* sp. at the same time of isolation, which was confirmed by serotyping morphologically different colonies from the same sample. This is in agreement with the study by Lastovica *et al.* (1986) where different campylobacter colonies from the primary isolation media were shown to be of different strains. The illness-to-infection ratio among the children decreased with increasing age, which was in accordance with other investigations (Glass *et al.* 1983; Calva *et al.* 1988; Taylor *et al.* 1988; Blaser *et al.* 1986). The study of Calva *et al.* shows that children at the age of 12–17 months had a diarrhoea-episode peak with 3·5 episodes/child/year. Glass *et al.* (1983) postulated an incidence rate for children living in Bangladesh of approximately 8·2 infections per child per year during the first 5 years of life.

The Swedish patients, on the other hand, with very few exceptions, were infected with one strain each time and reinfections were very rare. This pattern was found regardless of whether the patient was infected in Sweden or abroad.

The repeated infection episodes among the Mexican children, as a result of massive exposure is probably responsible for the high frequency of asymptomatic cases ($P < 0{\cdot}001$), as opposed to episodes associated with diarrhoea. This interpretation is in agreement with the longitudinal study from Calva *et al.*, where the illness-to-infection ratio was investigated in the same group of children. They concluded that symptom-producing early campylobacter infections seemed to protect against subsequent infection, whereas symptom-free infections do not (Calva *et al.* 1988). Asymptomatic episodes are less common or rare among people living in industrialized countries.

Comparing the serotypes common in Sweden with those common in Mexico, we found that for both HS and HL antigens, despite the wide serologic heterogeneity of *C. jejuni*, most clinical isolates belonged to relatively few serotypes. Similar findings have previously been reported from our own studies (Kaijser & Sjögren, 1985) as well as those of others (Patton *et al.* 1985).

Antigens (HS and HL) identified on strains from Mexican patients with symptomatic infections were, with few exceptions, the same as those isolated from patients without symptoms. HS-antigen serotype 37 was significantly ($P < 0{\cdot}05$) more common in symptomatic groups, and the HL-antigen serotype 9 ($P < 0{\cdot}05$) was more common in the asymptomatic group.

Several epidemiological studies have indicated that immunity against *C. jejuni* in humans is induced as a consequence of one or more infections (Glass *et al.* 1983; Calva *et al.* 1988; Jones *et al.* 1981). Blaser *et al.* (1983) have shown that persons who drink raw milk regularly and presumably have multiple exposures to *C. jejuni*, and have persistent elevations in anti-campylobacter immunoglobulin G levels. They show little or no campylobacter-related symptoms compared to

persons who drink raw milk for the first time. From these data the authors suggested that repeated exposure may lead to the acquisition of immunity. Svedhem *et al.* (1983) found that chicken slaughterhouse workers have an increased incidence of campylobacter antibody in serum as a consequence of daily exposure to campylobacter antigen. The workers reported that after an initial working-period with frequent diarrhoea episodes, they seldom fell ill with enterocolitis symptoms (personal communication). From the study of Calva *et al.*, there was a marked decrease in the illness-to-infection ratio with increasing age. Infants under 6 months of age had a ratio of 1:1, while infected children over 5 years of age did not have diarrhoea at all (Calva *et al.* 1988). In our present study on the Mexican children the finding of new serotypes in every reinfection might be compatible with a theory of induced immunity to surface antigen(s).

Several animal experimental studies have indicated the existence of homologous protective immunity by demonstrating the development of specific antibody responses to campylobacter infection (Ruiz-Palacios *et al.* 1983; Fitzgeorge *et al.* 1981; Burr *et al.* 1988).

Furthermore in studies among volunteers in the United States, Black *et al.* (1985) showed a natural immunity after repeated infections with *Campylobacter* sp. The study indicated the existence of a short-term homologous, protective immunity.

In conclusion we found that Swedish patients, who were mostly symptomatic, in general were infected with only one serotype of campylobacter and were rarely infected. Mexican patients, on the other hand, were in general infected with several serotypes and moreover were frequently reinfected with new, different serotypes. These results are in concordance with the development of an induced immunity to *C. jejuni* infection.

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