Serotypes of *Campylobacter jejuni* isolated from patients attending a diarrhoeal disease hospital in urban Bangladesh

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Summary. The serotypes of strains of *Campylobacter jejuni* isolated from patients attending the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B), Dhaka, and from animals were ascertained by Penner and Hennessy's (1980) serotyping scheme. Of 102 isolates from man, 74% were typable and serotypes 53, 15 and 22 predominated. Of 26 isolates from animals, 65% were typable and serotypes 15 and 53 occurred frequently. The diarrhoeal illnesses associated with different serotypes were similar. In one-third of cases other enteropathogens were present. *C. jejuni* was isolated from 7% of patients tested in a 4% sampling system during 1983, and the prevalent serotypes appeared in most months. It is concluded that *C. jejuni* is a common enteropathogen in Bangladesh, that a few serotypes predominate among isolates from both man and animals, and that serotype does not determine clinical symptoms.

Introduction

Soon after *Campylobacter jejuni* was recognised as an important cause of human gastroenteritis (Skirrow 1977), cases were reported mostly from Western Europe and North America. Reports of poultry and domestic animals as reservoirs (Blaser *et al.*, 1980b) and of possible transmission of the infectious agent via water, milk and food (Robinson *et al.*, 1979; Mentzing, 1981) have emphasised the need to characterise strains of this species. Moreover, the clinical features it produces are not identical everywhere (Blaser *et al.*, 1979; Billingham, 1981) and different serotypes predominate in different countries (Kaijser *et al.*, 1983). Because the organism is biochemically almost inactive and biotyping cannot differentiate between strains of the same species, several serotyping schemes have been developed. Two are well known—one (Penner and Hennessy, 1980) is based upon heat-extracted soluble antigens, and the other (Lior *et al.*, 1982) upon heat-labile antigens. Both schemes have been described as equally efficient for serotyping isolates from different countries (Kaijser *et al.*, 1983; Patton *et al.*, 1983).

In recent years, *C. jejuni* infections in tropical

countries have attracted interest, and reports of infections in man have come from Singapore (Lam, 1981), Thailand (Sabchareon *et al.*, 1982; Supavej *et al.*, 1982), Saudi Arabia (Choudhury *et al.*, 1981) and Nigeria (Low *et al.*, 1981). *C. jejuni* has been isolated with almost equal frequency from healthy individuals and from patients with diarrhoea in selected groups of the Bangladeshi population (Blaser *et al.*, 1980a). During 1979–1980, the rate of detection in patients attending the International Center for Diarrhoeal Disease Research (ICDDR) was 14% (Stoll *et al.*, 1982). It has been isolated along with other pathogens from six fatal cases (Butler *et al.*, 1983). It can produce an illness resembling shigellosis among travellers and expatriate residents (Speelman *et al.*, 1983), but such illness is uncommon in indigenous populations (Glass *et al.*, 1983). Serotyping has not been exploited in epidemiological and clinical studies (Blaser *et al.*, 1980a; Glass *et al.*, 1983; Stoll *et al.*, 1982) and, as a result, much about *C. jejuni* infection in Bangladesh remains obscure.

In the present study the method of Penner and Hennessy (1980) has been used to serotype isolates from man and animals in Bangladesh to determine the distribution of serotypes in patients, the value of prevalent serotypes as virulence markers, the seasonality of the disease, and its possible sources or origins.

Materials and methods

Isolation and sources of strains

Based on the registration number at ICDDR, B Treatment Centre, Dhaka, every twenty-fifth patient was included in the study from Jan. 1983 to Dec. 1983 inclusive. On admission, the patient's physical condition, including the state of hydration, was determined by a doctor, and the patient's guardian was interviewed by a trained health assistant to obtain a standard medical history (Stoll *et al.*, 1982). Weight and height were measured for assessment of nutritional status of patients under 10 years old by comparison with the Harvard Standard (Jelliffe, 1966). Stool specimens were screened for known enteropathogens except Norwalk agent and adenovirus.

To isolate and identify *Campylobacter* spp., fresh stools were cultured on Campy-BAP medium (BBL) in a candle jar at 42°C for 48 h as described by Blaser *et al.* (1980a). Characteristic colonies were tested for motility, H₂S production, and oxidase and catalase reactions, and stained by Gram's method for morphological examination. Isolates were confirmed as *C. jejuni* by testing their ability to hydrolyse hippurate (Harvey, 1980). The strains were stored at -70°C in Brucella Broth (BBL) containing glycerol 15%. In 1983, 204 isolates (one from each patient) were stored in serial order, and alternate isolates were serotyped. Twenty-six strains isolated from animals during another study in a rural area in 1981-1982 were also examined in this way.

Bacterial pathogens such as *Vibrio cholerae* O1, *V. cholerae* non-O1, *V. fluvialis*, *V. parahaemolyticus*, *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Shigella* spp., *Salmonella* spp. and *Yersinia enterocolitica* were identified by standard microbiological techniques (Edwards and Ewing, 1972; Lennette *et al.*, 1980). Heat-labile and heat-stable toxins of *Escherichia coli* were sought by the Chinese hamster ovary cell test and the infant mouse assay, respectively (Merson *et al.*, 1979). Rotavirus antigen was identified by enzyme-linked immunosorbent assay (Yolken *et al.*, 1977). *Giardia lamblia* and *Entamoeba histolytica* were identified by direct microscopy of unconcentrated faeces during routine examination.

Serotyping

The serotype of each strain was determined by detecting the saline-extracted thermostable antigens by the passive haemagglutination technique as described by Penner and Hennessy (1980) and Penner *et al.* (1983). Reference strains were obtained from Dr J. L. Penner, Department of Medical Microbiology, Faculty of Medicine, University of Toronto, Canada. Antisera to reference strains were prepared in rabbits by the method of Penner *et al.* (1980) except that Campy-BAP medium was used instead of blood agar to propagate the strains. Most of the sera had agglutinating titres of 2560-5120. These were used in the tests at an initial dilution of 1 in 100. Each isolate was tested against the panel of antisera

and assigned a serotype according to the antiserum in which agglutination occurred. When an isolate was agglutinated by more than one antiserum, the highest agglutinating titre was accepted as indicating the particular serotype.

Results

Serotypes of isolates from man

Of 102 strains, 75 were typable, four reacted equally with three different antisera, and 23 did not agglutinate with any antiserum. Although with many strains cross-agglutination occurred with two or three different antisera, the titre in cross-reactions was generally three- or four-fold lower than the highest titre obtained, on which basis the serotype was determined. Nineteen different serotypes were identified among the isolates from patients with a panel of antisera that could identify 42 specific antigens; the distribution of serotypes is shown in table I. Serotypes 53, 15 and 22 were dominant, constituting, respectively, 23.5%, 15.7% and 6.9% of the strains.

Clinical features

Three serotypes occurred most often among isolates from 31 patients in whom *C. jejuni* was the sole enteropathogen. The age and sex distribution in these patients and the clinical features associated with each serotype are shown in tables II and III respectively. The number of infections was highest in the age-groups under 1 year and 1-5 years; the sex ratio was 3:2 (M:F) and four patients only were malnourished. Among the clinical symptoms, a mild to moderate diarrhoea (mostly of watery stool), vomiting, mild dehydration and absence of fever were noteworthy. The duration of diarrhoea before coming to hospital was generally 2-4 days.

Table I. Distribution of serotypes among 102 clinical isolates

Serotype	Percentage of isolates
53	23.5
15	15.7
22	6.9
41	4.0
10, 33	3.0 (each)
5, 13, 21, 23, 38	2.0 (each)
1, 9, 16, 18, 19, 35, 36, 43	1.0 (each)
Untypable	22.5
Multireactive	4.0

Table II. Predominant serotypes related to age and sex of patients infected only with *C. jejuni*

Serotype	Total	Number of isolates in						
		age group (years)					sex	
		<1	1-5	6-12	13-25	>25	male	female
53	13	4	6	1	1	1	8	5
15	13	8	4	0	1	0	6	7
22	5	2	2	1	0	0	4	1

The three prevalent serotypes were isolated also from another 16 patients who had mixed infections with enteric pathogens such as enterotoxigenic *E. coli* (6 cases), *V. cholerae* O1 (5 cases), rotavirus (3 cases) and *S. flexneri* (2 cases). Infections in which *C. jejuni* was present with other pathogens were not restricted to specific age groups; five of the patients were malnourished. Among the patients with polymicrobial infections, five were moderately dehydrated and six were severely dehydrated from frequent purging of liquid stool.

Seasonality

From January to December inclusive, 2860 systematically selected patients were studied: *C. jejuni* was isolated from 204 of them. The figure

Table III. Clinical features in patients with *C. jejuni* as sole pathogen

Clinical feature	Percentage of patients with the given feature in infections with serotype		
	53 (n = 13)	15 (n = 13)	22 (n = 5)
<i>Frequency of stool</i>			
Low (3-5/day)	23	46	20
Moderate (6-10/day)	46	38	60
High (>10/day)	31	15	20
<i>Symptoms</i>			
Watery stool	85	92	80
Bloody stool	15	8	20
Vomiting	46	54	40
Mild fever (37.0-37.7°C)	15	23	0
Distended abdomen	30	15	20
<i>Dehydration</i>			
Mild	62	54	40
Moderate	23	13	40
Severe	0	8	0

Figure. The seasonal incidence of *C. jejuni* isolations in 1983.

shows that slightly more strains were isolated during the cold months of the year. Mixed infections with other enteric pathogens were found in 35% of cases in all months except April. Serotypes 15 and 22 were isolated in the months from March to September, whereas serotype 53 appeared in most months of the year.

Serotypes of isolates from animals

Twenty-six strains from 300 poultry and domestic animals were serotyped. Of the isolates from animals, 17 were typable, and serotypes 15 and 53 were most common (table IV). Serotypes 5, 15, 16, 25, 35 and 53 were found in household poultry or animals; all of them except serotype 25 were common in human patients.

Discussion

Although it was expected that nearly 95% of isolates would be typable by Penner's serotyping method, only 74% were typable. Three serotypes predominated among isolates from man. Cross-reactions, which were often encountered, may be due to the complexity and heterogeneity of *C. jejuni* antigens. It is likely that different serotypes predominate in different geographical localities.

In Bangladesh, the symptoms shown by patients from whom *C. jejuni* was isolated were mild to moderate purging of watery stool and mild dehydration, but fever was absent. Children under 5 years of age, of both sexes, were most often infected. The majority of Bangladeshi children suffering from *C. jejuni* enteritis were not malnourished. There were no significant differences between the clinical

Table IV. Distribution of serotypes in 26 poultry and pets

Origin	Number of strains of serotype							
	Total	5	15	16	25	35	53	Untypable
Chicken	16	1	3	0	1	0	3	8
Duck	2	0	1	1	0	0	0	0
Cat	1	0	1	0	0	0	0	0
Goat	3	0	0	0	0	2	0	1
Sheep	1	0	0	0	0	0	1	0
Cow	3	0	2	1	0	0	0	0
Total	26	1	7	2	1	2	4	9

features of illnesses associated with different serotypes of *C. jejuni* (tables II and III). It may be assumed, therefore, that *C. jejuni* produces mild diarrhoea, and that all clinical isolates cause similar disease irrespective of their serotypes. By contrast, patients who had polymicrobial infections including *C. jejuni* had moderate or severe diarrhoea. The severity of illness in multiple infection may be due to dominance of the other potential pathogen or to synergy. Dominance has been reported in some fatal cases (Butler *et al.*, 1983).

The 4% sampling system used in this study was found to be suitable for determining the seasonal pattern of various enteropathogens in a particular area (Stoll *et al.*, 1982). In the present study, although the incidence of *C. jejuni* infection was slightly higher in cold months, it did not show any clear seasonal trend; the monthly rate of isolation was in the range 4–15% with an average of 7%. Though serotype 53 was found throughout the year, serotypes 15 and 22 appeared only in hot months. In an earlier survey in rural Bangladesh, *C. jejuni* strains were isolated every month in almost equal

frequency (Glass *et al.*, 1983), but so far there has been no report of a campylobacter epidemic in Bangladesh.

Rearing of animals such as chickens, ducks, cats, dogs, sheep, goats and cows is common in rural Bangladesh. Serotypes 15 and 53, predominant in man, were common also in animals. Altogether six serotypes, five of which were common in urban patients, were found in rural poultry and pets (table IV). This may point to the possible origin of strains infecting man.

The somatic antigens of *C. jejuni* are heterogeneous. This limited study demonstrates that serotyping based on O-antigen determination is an excellent marker for *C. jejuni* strains but that serotype does not determine clinical symptoms.

We are grateful to Dr J. L. Penner for providing reference strains and to Dr D. A. Sack for guidance. We thank Mohammad Shahjahan Miah and Mr Priyatosh Sukul for their technical assistance. The study was supported by the Disease Transmission Programme, International Center for Diarrhoeal Disease Research, Bangladesh.

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