Epidemiol. Infect. (1997), **118**, 91–95

The serotype distribution of *Campylobacter jejuni* and *C. coli* isolated from patients with diarrhoea and controls at Tikur Anbassa Hospital, Addis Ababa, Ethiopia

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(*Accepted 12 October 1996*)

SUMMARY

Sixty-eight isolates of *Campylobacter jejuni* and *C. coli* isolated from patients with diarrhoea ($n = 630$) and controls ($n = 220$) at Tikur Anbassa Hospital, Addis Ababa, Ethiopia were serotyped on the basis of the heat-labile (HL) and the heat-stable (HS) antigens, by using 16 and 34 antisera, respectively, for the two methods. With the antisera against heat labile antigens, 89·3% of the *C. jejuni* and 75% of the *C. coli* were typable. The HL serotypes 1, 2, 3, 4, 5, 6 and 7 were the most common among the *C. jejuni* while HL serotypes 1 and 2 were dominant among the *C. coli* isolates. These serotypes accounted for 63·2% of all isolates. For the heat-stable antigens, 60% of the *C. jejuni* and 83·3% of the *C. coli* isolates were typable. The HS serotypes 1, 3, 8, 26 and 34 were most common among the *C. jejuni*, while serotypes 3 and 8 were dominant among *C. coli* isolates. This study shows that the most common HL and HS antigens among campylobacter isolates from Ethiopia correspond to the most frequent antigenic types from other parts of the world. A limited number of antisera were sufficient to identify the majority of the isolates.

INTRODUCTION

Since the recognition of campylobacter as one of the commonest causes of bacterial diarrhoea in humans [1–3], different typing systems such as serotyping [4–6], biotyping [7, 8], phage-typing as well as different genotypic methods [9, 11] have been proposed for improving the understanding of the epidemiological features of campylobacter diseases. The serotype distribution from different parts of the world [12–15] has been investigated using the Lior [4] and the Penner [5] system but not hitherto from Ethiopia. The aim of the present work was to study the frequency of different serotypes among campylobacter isolated from diarrhoeic patients and controls at Tikur Anbassa Hospital, Addis Ababa, Ethiopia.

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METHODS

Sources of campylobacter strains

All 68 isolates in this report came from a study including 630 patients with diarrhoeal disease of which 10·8% ($n = 66$) were positive for campylobacter. Of the 220 controls without symptoms of diarrhoeal illness, only 0·1% were positive for campylobacter. All samples were collected from patients from Addis Ababa and who attended Tikur Anbassa Hospital, Addis Ababa, Ethiopia between February 1992 and January 1993. Of the 630 patients, 232 were adults (15 or more years of age) and 398 were

children (less than 15 years of age). Children aged less than 1 year dominated among the patients and represented 42·7% of all, whereas the age group 15–34 years represented 24·7%.

Isolation and identification of strains

All stool specimens were obtained from defecated material and cultured directly on campylobacter blood-free selective agar (Oxoid Ltd, Basingstoke, Hampshire, England), which is selective for the isolation of *Campylobacter jejuni*, *coli* and *lari* [16]. The medium was supplemented with cefoperazone (Sigma Ltd, USA) 32 mg/l and crystal violet (Kebo, Sweden) 0·1%, 1 ml/l, to suppress the normal faecal flora. Cultures were incubated at 42 °C for 48 h in a microaerobic atmosphere which was achieved in anaerobic jars (Oxoid) with a palladium catalyst by using gas generating kits (Oxoid). The growth of *Campylobacter* species was confirmed by their characteristic appearance on culture media, gram staining reaction and positive tests for oxidase and catalase. All campylobacters isolated were kept frozen at −70 °C as stab cultures in 1% nutrient agar until species differentiation and serotyping were done.

Differentiation of the isolated *Campylobacter* species

The campylobacter isolated were defined as *C. jejuni* or *C. coli* by the rapid hippurate hydrolysis tests proposed by Lior and colleagues [8].

Serotyping assays

Heat-labile (*HL*) *antigens*

These were detected using the direct slide agglutination technique with whole, live bacteria as described by Lior and colleagues [4]. The absorbed polyclonal HL antisera used were anti-1, 2, 4, 5, 6, 7, 8, 9, 11, 12, 13, 17, 20, 21, 35 and 36 respectively, which have earlier typed 90% of strains in Swedish studies [12, 17] and are the antisera corresponding to the most common antigens in Canada [4].

Heat-stable (*HS*) *antigens*

These were detected using the indirect haemagglutination technique as described by Penner and colleagues [6], with a heated supernatant from the bacterial culture as antigen. The polyclonal HS antisera used were 1, 2, 3, 4, 5:1 (*C. jejuni*), 5:2 (*C. coli*), 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 20, 24, 26, 27, 28, 30, 31, 34, 37, 39, 46, 48, 49, 51, 56 and 59 which had earlier been shown to give a typability of 75% for *C. jejuni/coli* strains from Swedish patients. The corresponding antigens are common in Canada [6]. The definition of a non-typable strain in this study was a strain not typable with any of the 16 HL or 34 HS antisera used.

Reference strains

Reference strains for *C. jejuni* (NCTC 11351) and *C. coli* (LMG 6440) were used for quality control throughout the study.

RESULTS

Of the campylobacters that were differentiated at species level, *C. jejuni* accounted for 82·4% and *C. coli* for 17·6% of the isolates.

Heat-labile antigens

With the 16 antisera against heat-labile antigens, 59/68 (86·8%) of the campylobacter isolates were typeable, whereas the remaining 9 (13·2%) were untypable (Table 1). Of the 56 *C. jejuni* and 12 *C. coli* isolates, 50 (89·3%) of the *C. jejuni* and 9 (75%) of the *C. coli* were typable. A total of 11 serotypes were represented among the *C. jejuni* and 3 among the *C. coli* isolates (Table 1). HL-serotypes 1, 2, 4, 5, 6 and 7 were most common among the *C. jejuni*, while HL-serotypes 1 and 2 were dominant among the *C. coli* isolates. HL-serotypes 1, 2, 4, 5, 6 and 7 accounted for 63·2% of all isolates. Of the 56 *C. jejuni* 10 (17·9%) and of the 12 *C. coli* strains 2 (16·7%) were positive for more than one of the HL-serotypes as shown in Table 1. Serotypes 1 and 2 were common for both *C. jejuni* and *C. coli*, whereas the remaining serotypes were found mainly among the *C. jejuni* isolates.

Heat-stable antigens

With 34 antisera against heat-stable antigens, 43/68 (63·2%) of the campylobacter isolates were typable, whereas of the remaining isolates 20 (29·4%) were

https://doi.org/10.1017/S0950268896007315 Published online by Cambridge University Press

Table 1. Campylobacter jejuni *and* C. coli *isolated from patients with diarrhoea and controls serotyped on the basis of the heat-labile (HL) antigen and heat-stable (HS) antigen*

Serotype/ serogroup (HL)*	*C. jejuni* ($n = 56$) No.	%	*C. coli* ($n = 12$) No.	%	Serotype/ serogroup (HS)†	*C. jejuni* ($n = 56$) No.	%	*C. coli* ($n = 12$) No.	%
1	11	19·6	3	25·0	34	4	7·1	—	—
2	10	17·8	4	33·3	1	3	5·4	—	—
4	4	7·1	—	—	3	1	1·8	2	16·6
5	2	3·6	—	—	8	3	5·4	—	—
6	5	9·0	—	—	26	2	3·6	—	—
7	4	7·1	—	—	30	1	1·8	1	8·3
8	—	—	—	—	51	1	1·8	1	8·3
9	1	1·8	—	—	10	1	1·8	—	—
11	—	—	—	—	17	—	—	1	8·3
12	—	—	—	—	37	1	1·8	—	—
13	1	1·8	—	—	46	—	—	1	8·3
17	—	—	—	—	49	1	1·8	—	—
20	1	1·8	—	—	1 & 30	1	1·8	—	—
21	—	—	—	—	3 & 8	1	1·8	—	—
35	—	—	—	—	3, 8 & 34	3	5·4	—	—
36	1	1·8	—	—	3 & 34	2	3·6	—	—
1 & 2	4	7·1	1	8·3	3 & 39	1	1·8	—	—
1 & 6	1	1·8	—	—	3 & 51	—	—	1	8·3
2 & 5	2	3·6	—	—	4, 8 & 34	1	1·8	—	—
2 & 6	—	—	1	8·3	8 & 34	1	1·8	1	8·3
2 & 36	1	1·8	—	—	8 & 51	2	3·6	1	8·3
6 & 21	1	1·8	—	—	10 & 18	1	1·8	—	—
4, 5, 6, 9 & 36	1	1·8	—	—	14 & 51	1	1·8	—	—
					30 & 34	—	—	1	8·3
					34, 8 & 51	1	1·8	—	—
NT‡	6	10·7	3	25·0	NT‡	18	32·1	2	16·6
					ND§	5	8·9	—	—
Total	56	100·0	12	100·0	Total	56	100·0	12	100·0

* 1 *C. jejuni* serotype 2 and 1 *C. jejuni* serotype 4 were from controls.
† 1 *C. jejuni* serotype 34 and 1 *C. jejuni* serotype 3 and 34 were from controls.
‡ NT, non-typable isolates.
§ ND, not done.

untypable and 5 (7·4%) were not typed (Table 1). Of the 56 *C. jejuni* and 12 *C. coli* isolates, 33 (60%) of the *C. jejuni* and 10 (83·3%) of the *C. coli* isolates were typable. A total of 14 serotypes were represented among the *C. jejuni* and 7 among the *C. coli* isolates (Table 1). HS-serotypes 1, 3, 8, 26 and 34 were most common among the *C. jejuni*, while HS-serotypes 3 and 8 were dominant among the *C. coli* isolates. HS-serotypes 1, 3, 8, 26, 30, 34 and 51 accounted for 42·6% of all isolates. Of the 56 *C. jejuni*, 15 (26·7%) and of the 12 *C. coli* isolates, 4 (33·3%) were positive for more than one of the HS-serotypes as shown in Table 1. Serotypes 3, 8, 30, 34 and 51 were common for both *C. jejuni* and *C. coli*, whereas the remaining serotypes were found mainly among the *C. jejuni* isolates.

DISCUSSION

Major questions in investigations of campylobacter hitherto have been directed towards understanding the sources of campylobacter infection as well as the mode of transmission of the bacteria. Poultry appears to be a significant source of campylobacter [13, 18, 19]. Different serotyping systems have been developed to provide additional markers in the study of the

https://doi.org/10.1017/S0950268896007315 Published online by Cambridge University Press

epidemiological features of these organisms [4–7, 20–22]. Out of this, a great variety of surface antigen structures have been described, e.g. polysaccharides, lipopolysaccharides, proteins [23–25]. It has been shown that for the two dominating typing systems, proteins, mainly flagellar, constitute the HL antigen scheme [23, 24] and lipopolysaccharides the HS antigen scheme. For the former there are approximately 122 antigens recognized and for the latter approximately 70. Epidemiological investigations have shown that a limited number of serogroups dominate, which means that these are the most frequently found serotypes around the world, and also the most commonly found in outbreaks and sporadic cases of enteric campylobacteriosis [12–14, 18, 22]. In the course of this study, 68 isolates (56 *C. jejuni* and 12 *C. coli*) were serotyped using the methods of Lior and colleagues [4] and Penner and colleagues [6], with 16 HL- and 34 HS-antisera, respectively. Seventy-five to 90% typability can be achieved with these antisera and for routine purposes this is considered sufficient [12, 17]. The HL-serotypes 1, 2, 4, 6 and 7 were the most common among the *C. jejuni* isolates, and accounted for 59·6% of all isolates. For the heat-stable antigens, a total of 14 serotypes were represented among the *C. jejuni* and 7 serotypes among the *C. coli* isolates. HS-serotypes 1, 3, 8, 26, 30, 34 and 51 accounted for 42·6% of all isolates. To our knowledge there was no epidemic outbreak that could have influenced our results during the study period. Furthermore, the epidemiology of campylobacter infections in Ethiopia is unknown. These results also show that the most common HL and HS antigens among campylobacter isolated from Ethiopia belonged to the most frequent serotypes found in other parts of the world [4, 6, 14, 17, 18, 22]. However, serotypes HS2 and HS4, which in other countries frequently have been detected in both outbreaks and sporadic cases [14, 18, 26], are not prevalent among sporadic cases in Ethiopia. These serogroups have commonly been associated with handling and/or consumption of chicken and cattle [14, 19, 27]. From this study, as in a former one [13], we also found that children in developing countries frequently carry more than one campylobacter strain at one occasion. From approximately 20% of the cases more than one strain could be identified, both in regard to the species identification as well as to the serotyping [25]. We conclude that serotyping of campylobacter is mainly useful for epidemiological studies and it is obvious that a limited number of antisera can be used for serotyping most of the *C. jejuni* or *C. coli* strains common in most parts of the world [4, 6, 10, 17, 22]. In some cases combined serotyping for both heat-labile and heat-stable antigens is necessary. If a choice is to be made, typing for the heat-labile antigen seems simple and gives somewhat higher typability.

ACKNOWLEDGEMENTS

We would like to extend our heartfelt thanks to the staff and residents of internal medicine of Tikur Anbassa and the Ethio-Swedish Children's Hospitals for the continued support that they showed us in all aspects of our research activity. This work was supported partly by the grants available from the Swedish Agency for Research Cooperation with developing countries (SAREC) programme for Bio-Medical Research and Training.

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