Epidem. Inf. (1988), **100**, 227–237
Printed in Great Britain

High prevalence of campylobacter excretors among Liberian children related to environmental conditions

By KÅRE MØLBAK, NIELS HØJLYNG

Malaria Research Laboratory, Statens Seruminstitut, Amager Boulevard 80, 2300 Copenhagen S, Denmark

AND KNUD GAARSLEV

Department of Diagnostic Bacteriology, Statens Seruminstitut, Amager Boulevard 80, 2300 Copenhagen S, Denmark

(*Accepted 23 October 1987*)

SUMMARY

Campylobacter was the bacterial pathogen most prevalent in 859 children, aged 6–59 months, examined in a house-to-house diarrhoea survey in two Liberian communities. 44·9% of the children from an urban slum and 28·4% from a rural area were excretors. Since the prevalence of diarrhoea was very high and consequently many convalescent carriers were found, it was not possible to evaluate the pathogenic role of campylobacter.

The excretor rate increased with age and was significantly correlated to the use of supplementary feeding, inversely correlated to the quality of the water supply, and also associated with helminthic infestation. Results from re-examination of 172 children suggested a high intensity of transmission.

The findings all indicate the existence of a heavy environmental contamination with campylobacter, probably of both human and animal faecal origin.

INTRODUCTION

Since the description of a simple method for the isolation of campylobacter (Skirrow, 1977) this organism has been recognized as a major cause of acute diarrhoeal disease in developed countries (Svedhem & Kaijser, 1980; Blaser & Reller, 1981). In developing countries, where diarrhoea is a leading cause of early childhood morbidity and mortality (Walsh & Warren, 1979; Snyder & Merson, 1982), less is known about the clinical significance of campylobacter infection, although most studies have revealed widespread existence of campylobacter with varying prevalences (De Mol & Bosmans, 1978; Bokkenheuser *et al.* 1979; Blaser *et al.* 1980; Ringertz *et al.* 1980; Berry, Gracey & Bamford, 1981; Billingham, 1981; Low, Lawande & Hall, 1981; De Mol *et al.* 1983; Glass *et al.* 1983; Guerrant *et al.* 1983; Lloyd-Evans, Drasar & Tomkins, 1983; Olarte & Perez, 1983; Georges *et al.* 1984; Schneider, Parent & Maire, 1984; Rowland *et al.* 1985).

In contrast to developed countries, many subclinical infections occur, and in addition it has often been difficult to relate causally the demonstration of campylobacter to a particular episode of diarrhoea (Bokkenheuser *et al.* 1979; Blaser *et al.* 1980; Berry, Gracey & Bamford, 1981; Billingham, 1981; Glass *et al.* 1983; Olarte & Perez, 1983; Georges *et al.* 1984; Schneider, Parent & Maire, 1984; Rowland *et al.* 1985). Also differences in the age-specific rates of isolation suggest that the epidemiology is different in developed and developing countries (Blaser & Reller, 1981). Little is known about concomitant infections with other microorganisms and the role of hygienic and social factors.

In West Africa only few studies have been carried out (Billingham, 1981; Low, Lawande & Hall, 1981; Lloyd-Evans, Drasar & Tomkins, 1983; Schneider, Parent & Maire, 1984; Rowland *et al.* 1985), and none in Liberia. As a part of a major diarrhoea survey (Højlyng *et al.* 1985; Højlyng, Mølbak & Jepsen, 1986) we studied the prevalences of campylobacter and other pathogens in children aged 6–59 months residing in an urban slum area and a typical rural subsistence farming area.

MATERIALS AND METHODS

Area. Two areas, one an urban slum and the other a rural district comprising three villages, were selected for the study.

The urban slum, West Point, is a shanty town situated on a 0·25 km^2 peninsula extending from the capital city Monrovia into the Atlantic Ocean. The population, estimated as 30000, lived under very poor conditions, primarily in one-storey tin shacks. Most households had access to community standpipes, but facilities for waste disposal were almost non-existent. Domestic animals, predominantly hens and pigs, were present in certain parts of the slum. The community was served by one school and one public health clinic.

The rural district, Bong County, is situated 200 km inland in a forest area. The population in the three typical villages, approximately 3500, lived in adobe houses with no facilities. The main water supply was shallow wells, and in one third of the households the members used pit latrines. The number of domestic animals were lower than expected. A small health clinic and a school were functioning in each of two of the villages.

Households. A household was defined as an extended family living together and sharing a common cooking pot. Households with children aged 6–59 months participated in the study. The households were visited repeatedly until all children, residing more than 4 weeks in the household, were seen.

The refusal or fall-out rates were low in both areas, less than 4%. Information on the socioeconomic condition, housing, family size, ethnic group, water supply, personal and domestic hygiene, storage of precooked food, etc. were recorded.

Children examined. At West Point, 352 children from 185 households were examined, selected as follows: 284 children living in 144 households, randomly sampled from a house-to-house map (group A), 42 children with diarrhoea seen at the public health clinic at West Point (group B), and 26 children living in the same 41 households as group B, but not visiting the clinic (group C).

In Bong County, all children in the age group living in the three villages were examined, comprising a total of 518 children from 260 households.

All the children were examined physically, including weight and height, a medical history was recorded, and a blood smear and a stool sample were taken.

Diarrhoea. A child was considered to have diarrhoea only if the stool was watery or soft (i.e. taking the shape of the container used for collecting), and also the respondee claimed the child had diarrhoea.

Follow-up. A total of 194 non-randomly chosen children were examined 1 month later by the same procedure as on the first visit. Ninety-seven living in the urban area (85 belonging to group A, 11 to group B, 1 to group C), and 97 in the rural area. 172 of these children were examined for campylobacter.

Season. The study was carried out during the dry and late dry season, January–April 1983.

Examination of faecal specimens

Collection: A total of 1003 samples from freshly voided stools were collected in sterile containers, and kept in an insulated box with ice packs (5–10 °C) until cultured within 24 h. In addition 51 samples were taken by a 'rectal wash', using 4 ml sterile phosphate buffered saline (PBS, pH 7·2) in a plastic pipette. Before culturing, each voided specimen was suspended and diluted 1:1 in PBS.

Campylobacter. Faecal suspension of 10 μl was inoculated on Skirrows medium (Skirrow, 1977), and the plates were incubated microaerobically at 35 °C for 48 h in an anaerobic jar with activated Gas Generating Kit for Campylobacters and catalyst (Oxoid, Basingstoke, Hampshire, England). Colonies suggestive of Campylobacter were examined by phase contrast microscopy, and categorized as campylobacter when a typical curved or spiral rod exhibiting darting motility was seen. Eleven of the 352 children from West Point were not examined for campylobacter for technical reasons. Isolates were kept in beef extract agar at 35 °C, and within 1 month sent to Denmark by air. The strains that survived were freeze dried, and later serotyped using the method described by Penner (Penner & Hennesy, 1980).

Other bacterial pathogens. Primary cultivation for shigella, salmonella, *Yersinia enterocolitica*, *Vibrio cholerae* and enteropathogenic/enterotoxigenic *Escherichia coli* were done using methods of the Department of Diagnostic Bacteriology, Statens Seruminstitut, Copenhagen, seeding directly on media with intended low selectivity and high differentiating capacity (SSI medium). Isolated colonies were kept in beef extract agar and within 1 month sent to Statens Seruminstitut by air for verification and further identification.

From diarrhoeic children below 30 months of age, 0–7 morphologically different *E. coli*-like colonies were taken, and when confirmed biochemically, serotyped and tested at the International Center of *Escherichia* and *Klebsiella*, Statens Seruminstitut, for the production of heat-labile toxin (LT) by the Y-1 adrenal cell assay (Donta, Moon & Whip, 1974) and heat-stabile toxin (ST) by the suckling mouse assay (Dean *et al.* 1972).

Swabs were taken from all the fresh faecal samples, kept in Stuart's transport medium at 20–25 °C, and within 1 month cultured in and seeded from selenite enrichment medium at Statens Seruminstitut. Suspensions from the swabs were used to examine for rotavirus by enzyme-linked immunosorbent assay (Dakopatts, Copenhagen, Denmark).

Enteric parasites. Initial examination for enteric zooparasites were made without concentration by direct microscopy of the faecal suspension and of the faecal suspension diluted one to one in Lugols iodine. Faecal suspensions were then preserved by adding formalin (final concentration 4% formaldehyde) and later concentrated (Allen & Ridley, 1970). For the demonstration of *Cryptosporidium* oocysts a thick smear was made from the sediment and stained by the modified Ziehl–Neelsen technique (Henriksen & Pohlenz, 1981).

Statistical methods. Statistical evaluation was done by χ^2 with Yates correction, χ^2 trend analysis (Armitage, 1971) or Mantel–Haenszel–χ^2 (Kleinbaum, Kupper & Morgenstern, 1982). A P value below 0·05 was considered significant.

RESULTS

The relation of campylobacter-excretion to a number of factors was as follows:

Area. The results from the first examination are given in Table 1. There were no differences between the prevalence of campylobacter in the groups from West Point. The prevalence rate at West Point was 44·9% and in Bong County 28·4%. This difference is significant ($P < 0{\cdot}001$, χ^2–Mantel–Haenszel).

Age. The age-specific prevalences are shown in Fig. 1. In both areas studied, the prevalences were lowest (26·0% resp. 5·9%) in children aged 12–17 months, and stayed high in 3rd to the 5th year of life (51·3% resp. 34·0%).

Diarrhoeal disease. In Table 2 is given the prevalence of campylobacter excretion in children who had diarrhoea at the time of sample collection, a history of diarrhoea in the previous 2 weeks, or no diarrhoea during this period. Within each area, there were no differences observed.

Breast feeding. Table 3 shows the prevalence of campylobacter excretion in children fed only on breast milk, on combined breast milk and supplementary food, or on non-breast milk feeding. Children aged 6–11 months who were only breast fed had the lowest prevalence. The difference is statistically significant ($P < 0{\cdot}01$ for children 6–17 months of age, χ^2trend).

Water supply. Table 4 gives the frequency of campylobacter excretors among children in Bong County related to the water supply of their households. The prevalence rate was associated significantly with the type of the supply, being low (19%) among children from households with access to hand pumps, and higher among children from households with less controlled water supply ($P < 0{\cdot}01$, χ^2 trend). At West Point, 90% of the population used public standpipes. Among children from these households, the prevalence rate was 45·8%, compared with 38·2% in households with private water taps (not significant, χ^2).

Excretion turnover. A total of 172 children were re-examined for campylobacter 1 month after the first examination. As can be seen from Table 5, 53% of these children were positive on at least one occasion.

Excretion of other organisms. The prevalence of other enteric pathogens in children with and without campylobacter infection is shown in Table 6. Helminthic and protozoal infestations were very common. In the urban slum, 73% of the children each harboured from one to four helminths, the most common being *Trichuris trichiura* and *Ascaris lumbricoides.* In the rural area the prevalence was lower, 53%, each positive specimen containing from one to three different species, dominated by *A. lumbricoides* and hookworms. The helminthic species were more prevalent among children excreting campylobacter compared to the negative children. The difference was significant in all age groups from Bong County ($P <$

Table 1. *Prevalence of campylobacter excretors among children aged 6 months to 5 years*

Area	No. of children examined	No. excreting campylobacter	Excretors (%)
West Point	341	153	44·9
Group A	276	133	48·2
Group B	39	12	30·8
Group C	26	8	30·8
Bong County	518	147	28·4

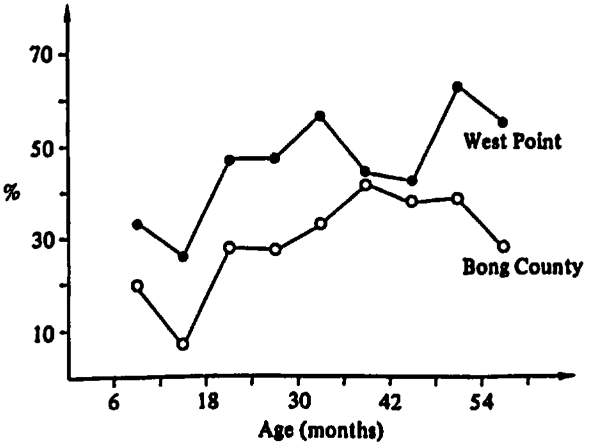

Fig. 1. Prevalence of campylobacter related to study area and age of children. Difference between areas: $P < 0{\cdot}001$, χ^2-Mantel-Haenszel.

Table 2. *Prevalence of campylobacter excretion in children with diarrhoea at the time of examination, with a history of diarrhoea within the previous 2 weeks period, and without both*

	West Point		Bong County	
Diarrhoea	No. of children examined	Excretors (%)	No. of children examined	Excretors (%)
Present	133	45	108	33
History of diarrhoea	93	41	234	26
No diarrhoea	112	46	169	28
No information	3	—	7	—

Table 3. *Campylobacter infection related to breast feeding*

	Prevalence of campylobacter in age groups					
Type of feeding	6–11 months		12–17 months		6–17 months	
Only breast	2/16	(13%)	0/2	(0%)	2/18	(11%)
Breast and supplementary	24/107	(22%)	6/69	(9%)	30/176	(17%)
Stopped breast	4/6	(67%)	9/29	(31%)	13/35	(37%)

Table 4. *Prevalence rate of campylobacter excretors in Bong County related to type of water supply*

Type of water supply	No. of children examined	Mean age (months)	Positive (%)
Stagnant water	163	31	35
Shallow well	123	33	32
Improved well	145	31	21
Hand pump	68	32	19
Unknown	19	—	—

Table 5. *Presence of campylobacter at first examination, and 1 month later*

First examination	Second examination	Children (n = 172) (%)
Positive	Positive	11
Positive	Negative	21
Negative	Positive	21
Negative	Negative	47

Table 6. *Prevalence of other enteric pathogens in children with and without campylobacter infection*

	Campylobacter excretion			
	West Point		Bong County	
	Positive	Negative	Positive	Negative
No. of children	153 (%)	188 (%)	147 (%)	371 (%)
Organism				
Shigella	2·0	2·7	2·7	2·7
EPEC/ETEC†	(27·3)	(18·4)	(16·7)	(31·1)
G. lamblia	16·3	10·1	14·3	15·1
Cryptosporidium‡	(5·4)	(8·2)	(4·8)	(9·7)
E. histolytica	2·0	1·1	2·7	3·0
Helminths	85·6*	63·3	66·0**	48·2
A. lumbricoides	47·1	34·0	40·1	32·9
T. trichiura	76·5	58·5	15·0	9·4
Hookworm	15·7	12·2	37·4	24·0
S. stercoralis	21·6***	10·1	2·7	0·5

† EPEC, Enteropathogenic *E. coli*; ETEC, Enterotoxigenic *E. coli*. From the two areas were 22 resp. 12 campylobacter excretors, and 49 resp. 45 campylobacter-negative children examined.

‡ 92 resp. 42 campylobacter excretors were examined for *Cryptosporidium* and 122 resp. 113 negative children.

* Compared with campylobacter-negative children, $P < 0{\cdot}01$, χ^2–Mantel–Haenszel.

** Compared with campylobacter-negative children, $P < 0{\cdot}025$, χ^2–Mantel–Haenszel.

*** Compared with campylobacter-negative children, $P < 0{\cdot}025$, χ^2–Mantel–Haenszel.

0·025, χ^2–Mantel–Haenszel), and in children below 3 years of age from the urban area ($P < 0{\cdot}01$, χ^2–Mantel–Haenszel).

Campylobacter excretion was not associated with infection by *Giardia lamblia*, *Cryptosporidium* (Højlyng, Mølbak & Jepsen, 1986), *Entamoeba histolytica*, shigella, or enteropathogenic and toxigenic types of *E. coli*.

Other factors. No association were found between prevalence rate of campylobacter and the following: type of excreta disposal, storage of prepared food, size of household, ethnic group, household income, social status, education and nutritional status. One fourth of the children were under-weight (Højlyng *et al.* 1985).

Identification of campylobacter spp. Twenty-two strains survived the transport to Statens Seruminstitut. All were thermophilic types, 11 being *C. jejuni*, five *C. coli*, two hippuricase-negative nalidixic acid-resistant thermophilic *Campylobacter* spp., and four had a non-conclusive hippuricase test. Eight (36%) of the strains were a serotypable: Three type pen 22, three pen 5, one pen 23 and one pen 37. Fourteen strains were non-typable.

There was no association between diarrhoea and specific species or serotypes.

DISCUSSION

This study is the first study in Liberia of the infectious agents commonly associated with diarrhoea in children. Campylobacter was the bacterial organism most prevalent in this study. Other investigators have also reported high excretion rates in developing countries: Bangladesh (Blaser *et al.* 1980; Glass *et al.* 1983), aboriginal communities in Australia (Berry, Gracey & Bamford, 1981), South Africa (Bokkenheuser *et al.*) and Central African Republic (Georges *et al.* 1984). These reported rates were usually lower than in the present study. Also in other West African countries, high prevalences were found (Billingham, 1981; Lloyd-Evans, Drasar & Tomkins, 1983; Schneider, Parent & Maire, 1984; Rowland *et al.* 1985).

Campylobacter was in the present study significantly more prevalent in the poor, crowded and contaminated urban slum where also diarrhoea was more prevalent compared with the rural area. It is known from other studies that 50% of the patients with campylobacteriosis still excrete campylobacter after 2 weeks, and some have positive stool cultures as long as 8 weeks after clinical disease (Karmali & Fleming, 1979; Blaser *et al.* 1980; Porter & Reid, 1980; Svedhem & Kaijser, 1980). The prevalence of diarrhoea was very high in the two underpriveleged communities studied: two-thirds of the mothers claimed that the children had had diarrhoea during the previous fortnight. It was thus not possible to evaluate the clinical significance and the pathogenic role of campylobacter from this study. Many of the apparently asymptomatic carriers were probably convalescent carriers, still excreting campylobacter, while other were 'healthy'. Many subclinical or asymptomatic infections are often found when children are living under conditions with a high faecal transmission, heavy environmental contamination, high prevalence of diarrhoea and hyperendemicity of known pathogens (Figueroa *et al.* 1983; Højlyng *et al.* 1985; Højlyng, Mølbak & Jepsen 1986).

There was a marked rise in the age-specific excretion rates after the age of 18 months. This is probably explained by the increasing contact at this age to the outside world and animals. The lowest prevalence of campylobacter was, however, among children aged 12–17 months, lower than the prevalence in the age group 6–11 months. This could be a reflection of long term excretion after primary campylobacter infection in early infancy, an age group not included in the present study. The observation is in contrast to reports from other developing countries such as Bangladesh, where the isolation rate was high (39%) in children aged 12–23 months, and subsequently falling (Blaser *et al.* 1980). In Brazil a predominance in children below 1 year of age was found (Riccardi & Ferreira, 1980), and in Mexico, the isolation rate was 15% in children aged 7–12 months and 11% in children aged 13–48 months (Olarte & Perez, 1983).

Campylobacter infection was associated with the use of supplementary feeding. This association is probably due both to ingestion of contaminated weaning diet (Højlyng *et al.* 1985), and probably also a decrease in protection from the breast milk.

The vehicle in some larger outbreaks of campylobacteriosis has been identified as water (Mentzing, 1981; Vogt *et al.* 1982; Palmer *et al.* 1983), and in Colorado, USA, the drinking of unprotected water was found to be a risk factor for endemic campylobacter infection (Hopkins, Olmsted & Istre, 1984). In the present study, campylobacter infection was inversely related to the quality of the water supply, and faecal contamination of water supplies of animal and human origin was very common (Højlyng *et al.* 1985).

Of the 172 children re-examined 1 month after the initial examination, 42% were campylobacter-positive on only one occasion, and 11% on both (Table 5). This suggests that in general the excretion period is short, and that consequently every child might have several campylobacter episodes per year. Only a longitudinal study will reveal this further.

Helminthic infestation was in both areas associated significantly with campylobacter excretion. This relation could not, however, for statistical reasons, be demonstrated among children at West Point above 2·5 years of age because as many as 94% of the slum children above this age harboured helminths. The association is probably explained simply by the shared variable: the poor hygienic conditions, including heavy faecal soil contamination. Each of the four helminths listed in Table 5 are known to be transmitted through an environmental soil stage. Another explanation of the observed polymicrobism could be a more specific intermicrobial synergism. Other studies (Ringertz *et al.* 1980; Glass *et al.* 1983; Kaldor *et al.* 1983; Melamed *et al.* 1985) have reported that mixed infections occur more frequently in patients with campylobacter-positive stools. Since the helminthic infections can be assumed to be chronic, it could be postulated that the presence of helminths directly or indirectly creates a niche for campylobacter.

Only 22 strains (7·3%) were recovered after storage and transport from the beef-extract agar, partly because of a frequently occurring contamination by other intestinal bacteria, created during the inoculation directly from primary plates, partly due to the long storage period. It is striking that a large proportion (14/22) of the surviving strains were non-serotypable.

In conclusion, this study from Liberia implies a widespread environmental contamination with campylobacter originating from a frequent human carriage and probably also from an animal reservoir.

This conforms with all the observed relations, e.g.: the very high prevalence of campylobacter in the urban slum and the relatively lower prevalence in the less crowded and cleaner rural area, the rise in age-specific prevalence after the age of

18 months concomitant to an increased contact with the environment, the rapid turnover of the excretion, the absence of association with diarrhoeal symptoms, the relative protection against infection by either the cleaner breast milk or a higher quality of the water supply, and finally with the association to helminthic infections known to be transmitted via an environmental soil stage.

These investigations were supported in part by grant no. 104 Dan 8/341 from the Danish International Development Agency. We thank Dr Aloysius P. Hanson, Director of the Liberian Institute for Biomedical Research, under whose auspices the survey in Liberia was carried out, and Dr Bertil Kaijser, Institute of Medical Microbiology, University of Gothenburg, Sweden, for the serotyping. Dr Nils H. Axelsen, Director of Statens Seruminstitut, Dr Søren Jepsen, Department of Parasitology, Drs Ida and Fritz Ørskov, International Center of *Escherichia* and *Klebsiella*, and Mr Severin Olesen Larsen, Department of Biostatistics, all Statens Seruminstitut, Copenhagen, are acknowledged for their contribution. Ms Gunild Frederiksen are thanked for the processing of the many specimens. Dr Kirsten Andersen participated in the planning of the project and in the fieldwork in Liberia.

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https://doi.org/10.1017/S0950268800067364 Published online by Cambridge University Press