

## **Campylobacter diarrhoea and an association of recent disease with asymptomatic shedding in Egyptian children**

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### SUMMARY

A hospital-based case-control diarrhoea survey was conducted in Cairo, Egypt to determine the age-specific frequency of campylobacter infection among diarrhoeic and non-diarrhoeic children aged new born to 5 years. Campylobacter was the most common bacterial enteropathogen isolated from diarrhoeic stools. The overall prevalence of campylobacter isolations was 25·9% from stools of 143 diarrhoeic children compared to 15·2% of 132 non-diarrhoeic control children ( $P = 0\cdot028$ ) during the 4-month period of study. Children less than 1 year of age were at greatest risk of campylobacter infection with 32·6% of diarrhoeic patients culture positive, compared to 14·3% of controls. Asymptomatic shedding in controls was positively associated with a recent diarrhoeal episode ( $P = 0\cdot019$ ) and may be an important source of new infections.

### INTRODUCTION

During the last decade, campylobacter has been shown to be an important cause of diarrhoea worldwide, affecting persons of all ages, in both industrialized and developing nations [1, 2]. For infants and young children of tropical developing countries, campylobacter-associated diarrhoea is a major health problem, contributing substantially to childhood morbidity and mortality. In addition to its role as an agent of endemic diarrhoea, campylobacter is also an important cause of travelers' diarrhoea for visitors to developing countries.

One of the epidemiological features of campylobacter infections in developing countries is the frequently high prevalences of asymptomatic carriage in children. In Bangladesh [3] and India [4], almost 40% of healthy children less than 2 years

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of age have been shown to harbour the organism. Despite a significant association with diarrhoeal disease in younger age groups, the high frequencies of asymptomatic campylobacter infection suggest these organisms are not the etiological agents for all episodes in which they are isolated, and that acute infections are frequently followed by periods of asymptomatic shedding.

Campylobacter-associated diarrhoea has not been extensively investigated in Egypt or other countries of North Africa or Southwest Asia. We conducted a hospital-based diarrhoea survey in Cairo, Egypt to determine the age-specific prevalence of Campylobacter isolation from stools of diarrhoeic and healthy children age newborn to 5 years. The study was performed during May through August, the peak diarrhoea season at the paediatric outpatient clinic of the study hospital.

## METHODS

### *Study population and specimens*

Fresh stool specimens were obtained from 143 diarrhoeic children ( $\bar{x}$  age = 14.2 months) during their attendance outpatient paediatric clinic in a large urban hospital. The study population, sampled from all available cases, were selected sequentially according to the availability of an interviewer, compliance to case definition, and laboratory capabilities. Diarrhoeic children (cases) were defined as those patients with a principal complaint of diarrhoea, who according to their mothers' had passed at least three diarrhoeic stools during the previous 24 h, and whose current stool specimen conformed to the shape of the specimen container. Diarrhoeic children with a recent history of antibiotic usage were not enrolled.

Rectal swabs from a comparable group of 132 apparently healthy children ( $\bar{x}$  age = 14.3 months), brought to the hospital for postnatal examinations, immunizations, or minor complaints, provided the comparison group (controls). Children whose mother reported that they were currently suffering from diarrhoea, or whose rectal swab indicated the possibility of diarrhoea, were excluded. Mothers were interviewed for a recent history of diarrhoea for each child.

Stool and rectal swab specimens were transported to the field laboratory in modified Cary–Blair medium [5], and processed within 4 h.

### *Laboratory identification*

*Campylobacter.* Specimens were inoculated onto modified Skirrow medium [6] and incubated at 42 °C in a microaerophilic environment (5% O<sub>2</sub>). Cultures were evaluated after 48 h; suspect colonies were identified as *Campylobacter* spp. based on colonial and gram stain morphology, motility, positive oxidase and catalase reactions, and sensitivity to nalidixic acid [3].

*Other bacterial enteropathogens.* For detection of *Salmonella* spp., *Shigella* spp., *Vibrio* spp., and *Plesiomonas* spp., faecal specimens were inoculated directly onto conventional enteric media and into selenite-F enrichment broths according to accepted standard procedures of enteric bacteriology [7]. Primary cultures and enrichment broths were incubated overnight at 35 °C. Enrichment broths were subcultured and evaluated after an additional 16–18 h growth. Suspect enteropathogens were identified using conventional biochemical tests, API 20E (Analytab Products, Plainview, NY), and commercial antisera (Difco, Detroit,

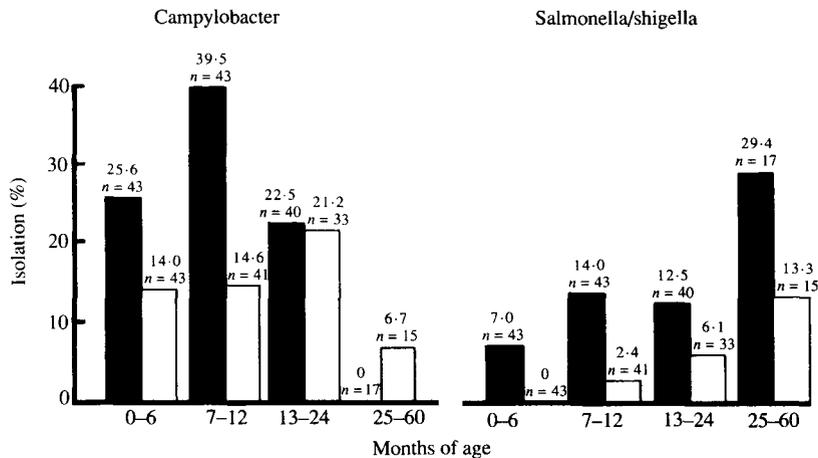


Fig. 1. Prevalence of bacterial enteropathogens, by age, for diarrhoeic and non-diarrhoeic children in Cairo, Egypt. ■, Cases; □, controls.

MI) when indicated. For isolation of *Yersinia enterocolitica*, specimens were inoculated into Christensen media, incubated for 21 days at 4 °C, and then subcultured and incubated for an additional 48 h at 28 °C [8].

## RESULTS

During the 4-month study, hospital records showed that 62.3% of the 1012 children presenting at the hospital clinic had diarrhoea as their principal complaint. Among those selected for study, *Campylobacter* spp. was isolated from 25.9% of cases and 15.2% of controls ( $P = 0.028$ ). *Salmonella* spp. or *Shigella* spp. were the only other significant causes of diarrhoea and have been grouped together in the current analysis. Combined, *Salmonella* spp. or *Shigella* spp. were isolated from 13.3% of cases and 3.8% of the control group ( $P = 0.005$ ). There were no isolations of *Yersinia* spp., *Plesiomonas* spp., or *Vibrio* spp. from diarrhoeic or control patients.

For diarrhoeic children, age specific isolation frequencies (Fig. 1) indicated the group with the highest risk of campylobacter-associated diarrhoea was 7–12 months of age. Risk appeared to decline significantly with age, although the number of children studied in the outlying age group was small. The age curve for campylobacter isolations in control children peaked during the second year of life, also sharply declining thereafter. The curve of salmonella shigella isolations was markedly different, showing an almost linear trend of increasing risk of infection with increasing age for both diarrhoeic and control children.

Monthly isolation frequencies (Fig. 2) showed campylobacter to be most commonly associated with disease in July when almost half of diarrhoeic children had this pathogen isolated from this stool. The frequency of asymptomatic carriage appeared to increase linearly over the period studied, with the highest observed frequency in the last month of study (August), which also marked the end of the diarrhoea season. In contrast, salmonella/shigella infections were most prevalent early in the season (May).

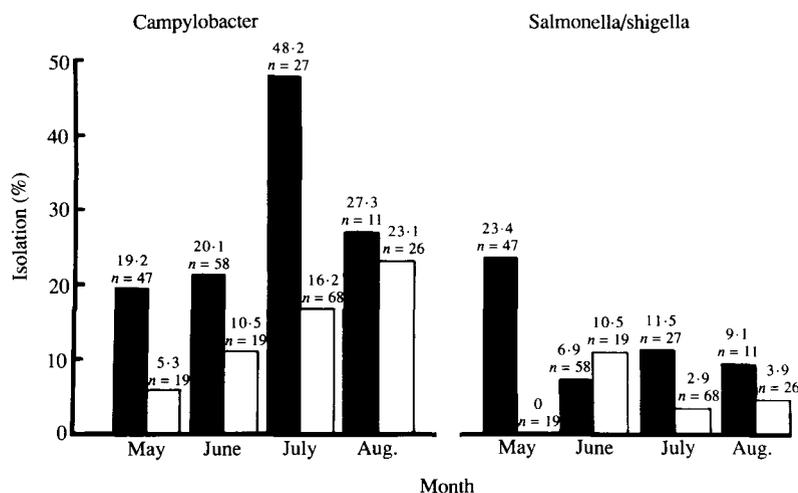


Fig. 2. Prevalence of bacterial enteropathogens, by month, for diarrhoeic and non-diarrhoeic children 5 years of age or less, in Cairo, Egypt. ■, Cases; □, Controls.

Histories from control children indicated that attacks of diarrhoea frequently preceded asymptomatic shedding of campylobacters, which were presumably the cause of the diarrhoea in many cases. Campylobacter was recovered from 30.3% of specimens from control patients with a recent history of diarrhoea (prior 7 days) compared with 9.3% of control subjects without a recent history of diarrhoea ( $P = 0.019$ ). There were no significant differences in isolation frequencies of salmonella/shigella among control children with a recent history of diarrhoea, compared to those without (3.0 and 2.3%, respectively).

#### DISCUSSION

The results indicate campylobacter infection is a major cause of diarrhoea in children of Cairo, Egypt during their first year of life. The prevalence of this organism in stools of diarrhoeic children of this age group (32.6%) is among the highest reported for pediatric surveys. The frequency of asymptomatic shedding (14.3%) was higher than that found in children of some developing countries [9–11], but less than Bangladesh [3] or India [4].

Campylobacter is frequently described as an important cause of diarrhoea in children less than 5 years of age. In Egypt, as in Bangladesh [3, 12] and India [4] where the organism is also highly prevalent in the population, the risk of disease appears to be predominantly during the first year of life. In the current study, although campylobacter isolations were frequent in 13- to 24-month-old children (22.5%), non-diarrhoeic children in this age group harboured the organism at almost the same frequency (21.2%). This indicates that, in an area where there is a constant high level of exposure, most children will have been infected and possess a significant amount of acquired immunity by 1 year of age.

An association between campylobacter isolation from healthy children and recent history of diarrhoea has been reported from a survey of Bangladeshi children where 48% of culture-positive asymptomatic children had diarrhoea in

the prior month, compared to only 20% of culture-negative matched controls [3]. A similar association was shown in the current study when campylobacter was cultured more often (30%) from stools of control subjects who had a recent history of diarrhoea, compared to control subjects without a recent episode (9%). The similar observations suggest that, in developing countries, 20–30% of children with campylobacter diarrhoeal episodes continue to shed the organism for 1–4 weeks following clinical recovery. They also suggest that asymptomatic children, who shed the organism in large numbers, may be an important reservoir and source of new infections.

The relationship between acute and asymptomatic states in campylobacter infected individuals also might have influenced local temporal trends of prevalence. In the current study, the peak frequency of isolation for campylobacter in diarrhoeic children occurred in July. As might have been predicted, the peak month for isolations in control children occurred in the following month, suggesting a significant proportion of the asymptomatic shedding was due to recovery from campylobacter-associated diarrhoea in the previous month. From a community perspective, such a phenomenon might be likened to a 'wave' of new infections passing through a population of susceptibles, followed by a 'wave' of asymptomatic shedders among the healthy population in the following month. Had the study continued, it is likely the month of September would have marked a decline in campylobacter isolations among asymptomatic children, reflecting the decline in the frequency of isolation among cases in the previous month. Because Egyptian children less than 1 year of age appear to be most susceptible to disease from campylobacter infection, such waves may occur on an annual basis during the summer diarrhea season in Cairo.

The evidence for frequent prolonged asymptomatic shedding following an acute episode of campylobacter-associated diarrhoea point out the need for careful consideration of diarrhoeal disease histories when conducting surveys of young children in developing countries which are aimed at assessing disease risk or transmission factors of campylobacter-associated diarrhoea. Studies of longer duration with greater attention devoted to discriminating between asymptomatic colonization and the convalescent shedding which follows an acute episode might contribute toward a better understanding of the epidemiology of campylobacter infections in developing countries.

In summary, these data indicate that campylobacter infection among children in Cairo is strongly associated with disease in children less than 1 year of age, that high levels of asymptomatic shedding occurs in children less than 2 years of age, and that the risk of campylobacter-associated diarrhoea, in children, is greatest in July. Indirect evidence suggest campylobacter-associated diarrhoea episodes are followed by periods of asymptomatic shedding following recovery which may influence temporal trends of prevalence, in addition to being an important source of new infections.

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