**Campylobacter Infection in Children in Malawi Is Common and Is Frequently Associated with Enteric Virus Co-Infections**

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### Abstract

**Background:** Campylobacter species are the most common cause of bacterial gastroenteritis in the developed world. However, comparatively few studies have determined the epidemiological features of campylobacteriosis in resource-poor settings.

**Methods:** A total of 1,941 faecal specimens collected from symptomatic (diarrhoeic) children and 507 specimens from asymptomatic (non-diarrhoeic) children hospitalised in Blantyre, Malawi, between 1997 and 2007, and previously tested for the presence of rotavirus and norovirus, was analysed for *C. jejuni* and *C. coli* using a real time PCR assay.

**Results:** Campylobacter species were detected in 415/1,941 (21%) of diarrhoeic children, with *C. jejuni* accounting for 85% of all cases. The median age of children with Campylobacter infection was 11 months (range 0.1–55 months), and was significantly higher than that for children with rotavirus and norovirus (6 months and 7 months respectively; P < 0.001). Co-infection with either rotavirus or norovirus was noted in 41% of all cases in the diarrhoeic group. In contrast, the detection rate of Campylobacter in the non-diarrhoeic group was 14%, with viral co-infection identified in 16% of children with Campylobacter. There was no association between Campylobacter detection rate and season over the 10 year period.

**Discussion:** Using molecular detection methodology in hospitalised Malawian children, we have demonstrated a high prevalence of Campylobacter infection, with frequent viral co-infection. The burden of Campylobacter infection in young African children may be greater than previously recognised.

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**Introduction**

It is estimated that 3.552 million children under the age of five die each year in Africa; diarrhoeal disease accounts for 11% of these deaths [1]. Diarrhoea also contributes to childhood morbidity, particularly malnutrition and growth stunting [2,3]. The introduction of intervention strategies, such as provision of oral rehydration therapy, has had a positive impact on reducing diarrhoeal deaths in Africa since 2000 of 3.7% [1]; however diarrhoea remains a leading cause of child mortality and morbidity in this region. The importance of pathogens such as rotavirus and enterotoxigenic *Escherichia coli* in the aetiology of severe childhood diarrhoea in developing countries is well recognised [4,5]. However the role of *Campylobacter* is less well understood.

*Campylobacter* is a fastidious gram negative bacterium and *C. jejuni* and *C. coli* are considered the most common cause of bacterial gastroenteritis in the developed world. The clinical presentation ranges from mild watery to severe inflammatory diarrhoea which may be complicated by post infectious sequelae such as Guillain-Barré Syndrome [6]. Using bacterial culture methodology, estimates of the prevalence of *Campylobacter* infection in young children with diarrhoea in Sub Saharan Africa range from 1.5% to 18% [7,8,9,10,11]. While molecular techniques have been developed and employed for *Campylobacter* detection in epidemiological studies in developed countries, such methods have not been widely adopted in Sub Saharan Africa. Where molecular detection (notably PCR) was used to examine for *Campylobacter* species in adults and children with diarrhoea in South Africa the prevalence estimates of *C. jejuni*, *C. coli* and *C. concisus* were 12%, 7.5% and 2.7% respectively; however only 34 of the 255 samples analysed were from children <5 years of age [12].

As part of a long-term research programme investigating viral gastroenteritis in children in Malawi, we have collected stool samples since 1997 from children <5 years of age admitted to the Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi.
with moderate to severe diarrhoea [5]. We have now examined stored faecal specimens using a real time PCR assay to determine the prevalence and epidemiological features of Campylobacter infection in this population.

**Methods**

**Ethics Statement**

Written, informed consent was obtained from the child’s parent or guardian prior to enrolment. Ethical approval was obtained from the Malawi National Health Sciences Research Committee.

**Study Site**

The QECH is a large government run tertiary referral hospital in the southern Malawian city of Blantyre, which has a population of approximately 1 million living in urban and peri-urban settlements.

**Specimen Collection**

Enrolment and data collection procedures have previously been described in detail [5]. Briefly, children age <5 years admitted to the QECH with ≥3 loose or watery stools within a 24 hour period for <14 days, were eligible for inclusion. Children were enrolled Monday to Friday, 9 am to 5 pm, from 1st July 1997 to 30th June 2007. A second group of children <5 years of age without diarrhoea, who were admitted to the QECH with conditions such as malaria and respiratory infections, were enrolled between 1997 and 1999. A single faecal specimen was obtained from each child.

Clinical data (illness severity, blood in stools etc.) were not routinely gathered. Following EIA testing for rotavirus [5], remaining samples were shipped to the University of Liverpool and stored at −80°C until testing for norovirus by real time PCR [13] and for Campylobacter (this study).

**Campylobacter Testing**

Of 2,458 faecal specimens collected from hospitalised diarrheic children in the primary study [5], 1,941 were available for testing for Campylobacter infection, together with 507 samples from children admitted to hospital without diarrhoea. DNA was extracted from all samples using an automated extractor (Qi asymphony, Qiagen). The presence of a 95 base pair fragment of the napA gene of C. jejuni and a 103 base pair fragment of the cceE gene of C. coli were detected using a real time PCR method [14].

**Statistical Analysis**

Data were analysed using “IBM SPSS Statistics Data Editor” version 11. Categorical data were analysed using Chi² test and continuous data using paired T-tests. A p-value of <0.05 was considered significant.

**Results**

**Characteristics of the Study Population**

In total 2,448 samples was analysed; 1,941 from diarrheic children and 507 from non-diarrheic children. The median age of children in the diarrheic group was 9 months (range 0–55 months) and in the non-diarrheic group was 6 months (range 1–50 months). The diarrheic group contained 55% males and the non-diarrheic group 52% males.

**Prevalence**

Over the 10 year study period (1997–2007) Campylobacter was detected in 415/1941 (21%) of diarrheic specimens. For the two year period (1997–1999) in which faecal specimens from non-diarrheic children were collected, the detection rate of Campylobacter was significantly higher in the diarrheic specimens than the non-diarrheic specimens (28% vs. 14%; p<0.001) (Table 1). Of the Campylobacter species detected between 1997 and 1999, C. coli comprised 10% and 4% of all Campylobacter from diarrheic and non-diarrheic specimens respectively (p<0.001). There was no statistical difference in the Campylobacter cycle threshold values obtained from diarrheic vs. non-diarrheic specimens (data not shown).

**Age Distribution**

The median age of children with diarrhoea in whom Campylobacter was detected was 11 months (range 0.1–55 months) which was higher than the age of children with rotavirus or norovirus (median age 6 months and 7 months respectively (p<0.001)). The detection rate of Campylobacter was relatively constant across all age groups, in contrast to the detection rate of rotavirus and norovirus which decreased with age (Figure 1). The median age of non-diarrheic children with Campylobacter infection was 11 months (range 1–34 months); the detection rate increased from 5% of children in the 0–2 month age group to 20% of children age >18 months (Table 1).

**Viral Co-infections**

In the diarrheic group 40% of children with Campylobacter had an enteric virus co-infection. These co-infections occurred predominantly in children <1 year of age with 50% of all Campylobacter cases in this age group also having either rotavirus or norovirus in the specimen. In the non-diarrheic group 16% of children with Campylobacter in the specimen also had a viral co-infection (Table 1). Although the overall prevalence of Campylobacter was significantly higher in the diarrheic than in the non-diarrheic group, when single infections were considered (i.e. in the absence of either rotavirus or norovirus) this difference is less pronounced (Campylobacter detection rate of 16% in the diarrheic vs. 11% in the non-diarrheic group, p=0.02).

**Seasonality**

The detection rate of Campylobacter did not vary consistently by month or season of specimen collection during the 10 year study period. In total 49% of Campylobacter positive specimens in the diarrheic group occurred in the dry season (May to October) and 51% in the wet season (November to April). In the non-diarrheic group 41% and 59% of positive specimens occurred in the dry and wet season respectively (p=0.89; data not shown).

**Discussion**

In this large study of Campylobacter infection in Malawian children, using a sensitive molecular assay we documented Campylobacter in 21% of specimens obtained from children <5 years of age hospitalised with diarrhoea. There are no previous data describing the prevalence of Campylobacter infection in Malawi. Estimates of the prevalence of Campylobacter infection in hospitalised children <5 years of age with diarrhoea in Sub Saharan Africa range from 1.5% in Botswana [12], 1.7% in Mozambique [8], 9% in Uganda [7], 11% in The Central African Republic [9] to 18% in Tanzania [10]. In community settings the detection rate of Campylobacter in diarrhoeal faecal specimens among children <5 years of age has been estimated at 15.9% in The Central African Republic [15], 3.3% in Djibouti [16] and 0.8% in Guinea-Bissau [17]. This variation in detection rate across Sub Saharan Africa may reflect the technical difficulties of isolating Campylobacter.
species in resource poor settings because of its fastidious growth requirements and/or the relative insensitivity of some culture techniques.

Comparing culture and PCR in the detection of *C. jejuni* and *C. coli* in diarrhoeal faecal specimens in Ireland suggested that culture alone detected only 55% of all cases [18]. In the UK, *Campylobacter* detection rates in community diarrhoea cases increased from 4.0% by direct culture, to 5.0% after a faecal enrichment procedure was added, to 15% by PCR [19]. A study in South Africa of 255 adults and children with diarrhoea and 67 children without diarrhoea, demonstrated similar overall rates of *Campylobacter* using PCR to those reported in the current Malawi study (19.6% in symptomatic patients and 11% in asymptomatic patients) [12]; however the prevalence in children <5 years of age with diarrhoea was 11% compared with 21% in the current study. Thus our data suggest that the detection rate of *C. jejuni* and *C. coli* using a sensitive and specific PCR assay in faecal specimens of children <5 years of age hospitalised with diarrhoea in Sub Saharan Africa may be higher than previously reported.

Of note, we documented a relative over-representation of *C. coli* compared with *C. jejuni* in the faecal specimens of diarrhoeic compared with non-diarrhoeic children. A similar pattern was reported in Tanzania where *C. coli* was present in 20% of diarrhoeic and 6% of non-diarrhoeic specimens from adults and

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**Table 1. Age Distribution of Campylobacter infection.**

| Age Group       | Total | Campylobacter positive | Campylobacter+virus | Campylobacter alone |
|-----------------|-------|------------------------|---------------------|---------------------|
| Diarrhoeic (1997–2007) |       |                        |                     |                     |
| 0–2 months      | 156   | 31 (20%)               | 20 (13%)            | 11 (7%)             |
| 3–5 months      | 412   | 83 (20%)               | 43 (10%)            | 40 (10%)            |
| 6–11 months     | 673   | 153 (23%)              | 70 (10%)            | 83 (12%)            |
| 12–17 months    | 418   | 82 (20%)               | 28 (7%)             | 54 (13%)            |
| >18 months      | 282   | 66 (23%)               | 7 (2%)              | 59 (21%)            |
| Total: 1997–1999 | 738   | 206 (28%)              | 87 (12%)            | 119 (16%)           |
| Non-diarrhoeic (1997–1999) |       |                        |                     |                     |
| 0–2 months      | 140   | 7 (5%)                 | 0                   | 7 (5%)              |
| 3–5 months      | 104   | 14 (13%)               | 0                   | 14 (13%)            |
| 6–11 months     | 81    | 15 (19%)               | 6 (7%)              | 9 (11%)             |
| 12–17 months    | 80    | 12 (15%)               | 2 (3%)              | 10 (13%)            |
| >18 months      | 102   | 21(21%)                | 3 (3%)              | 18 (18%)            |
| Total: 1997–1999 | 507   | 69 (14%)               | 11 (2%)             | 58 (11%)            |

χ² (Diarrhoeic vs. non-diarrhoeic 1997–1999) = 35.7

| ρ value          |
|------------------|
| p value          |
| <0.001           |
| <0.001           |
| 0.02             |

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**Figure 1. Age distribution of Campylobacter, rotavirus and norovirus infections in Malawian children with acute diarrhoea, 1997–2007.**
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Campylobacter was detected in 14% of non-diarrhoeic children who were recruited over a two-year period. Since no preceding clinical or microbiological data are available for these children, we are unable to determine whether this finding represents asymptomatic colonisation or extended excretion after resolution of a Campylobacter diarrhoeal episode. Asymptomatic colonisation appears to occur frequently in children in Sub Saharan Africa; one longitudinal study reported that 41.7% of all children in a community birth cohort were asymptotically colonised with Campylobacter within the first 6 months of life [13]. This phenomenon does not appear to occur in the developed world with the exception of workers occupationally exposed to Campylobacter [28]. It is thought that regular exposure to Campylobacter within an abattoir or farm environment results in immune tolerance towards Campylobacter species and hence facilitates colonisation [29]. It is feasible that a similar response occurs in children in Sub Saharan Africa explaining the observed high rates of asymptomatic infection. Given the high sensitivity and specificity of the assay used in this study it is also possible that the detection rates seen in the control cohort could represent post excretion following a diarrhoeal episode as Campylobacter is known to be excreted for up to 12 weeks post infection [30].

This study highlights specific differences in the epidemiology of Campylobacter in developed compared to developing countries. Firstly, in the UK, the detection rate of Campylobacter in children with and without diarrhoea is less than that seen in children in Sub Saharan Africa (6.8% and 2% in diarrhoeal and non-diarrhoeal children in the UK respectively) [14]; secondly, there are age specific differences in detection rates in children in the UK with prevalence increasing throughout childhood [14]; and lastly there is a strong seasonal association in temperate climates with a peak in incidence occurring during the warmer months whereas no seasonal association was seen in Malawi [31]. These differences may be explained by a number of factors including differences in the routes of transmission between the two settings; in the developed world the majority of Campylobacter strains causing human infections can be epidemiologically linked to strains that colonise poultry, with the main route of transmission of the pathogen thought to be through the handling and consumption of contaminated meat [32]. Although there are no comparative epidemiological studies linking poultry and human strains in Sub Saharan Africa, up to 40% of commercial chickens in Senegal and 60% in South Africa are colonised with Campylobacter [33,34]. Furthermore, strains colonising chickens in Senegal are genetically similar to strains colonising chickens in the UK, The Netherlands and United States suggesting that the host association of Campylobacter genotypes transcends geographical boundaries [35].

Chicken is a widely consumed meat in Malawi and many families keep chicken flocks which are routinely housed within the human dwelling including in food preparation areas [36]. Thus in contrast to the developed world, there is increased potential for acquisition and spread of zoonotic and foodborne disease such as Campylobacter which may account for the higher prevalence rates reported in this and other studies.

In conclusion, this large study of children hospitalised with diarrhoea in Malawi suggests that the burden of Campylobacter is higher than previously appreciated, and is frequently identified in association with concomitant rotavirus and/or norovirus infection. Given the recent introduction of rotavirus vaccines into childhood immunisation programmes in Malawi and other parts of Sub Saharan Africa it is predicted that bacterial pathogens including Campylobacter could play a more prominent role in the aetiology of diarrhoeal disease in young children in this region in the future. An improved understanding of the clinical features, epidemiology,
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