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Brief Communication

Detection and species identification of *Campylobacter* in stool samples of children and animals from Vellore, south India

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Abstract

Campylobacter spp. are an important cause of bacterial gastroenteritis frequently isolated from animal, poultry and environmental samples. In this study, we investigated the zoonotic potential of *Campylobacter* spp. by comparing prevalence rates and species in 394 children with diarrhoea and 652 animals in Vellore using PCR-based tools. Eighteen children (4.5%) had campylobacteriosis, a majority of whom had co-pathogens (15/18) and most were infected with *Campylobacter jejuni* (16/18). A few *C. coli* and mixed infections with both species were also seen. Among the animal samples, 16/25 chicken samples (64%) were positive and all were found to be *C. jejuni*.

Key words: *Campylobacter, chicken, gastroenteritis, zoonotic*

Introduction

Campylobacter spp. are one of the most common causes of bacterial gastroenteritis with human campylobacteriosis caused principally by *Campylobacter jejuni* and *C. coli* and occasionally by *C. lari*. *Campylobacter* gastroenteritis is especially common in children during the first 5 years of life with reported isolation rates of up to 46%.[1] This pathogen is also associated with post-infectious auto-immune sequelae including Guillain-Barré syndrome. In developed countries, consumption of contaminated chicken, red meat, water, milk, and contact with pets and farm animals have been implicated as potential sources of *Campylobacter* infection. The precise extent of the risk posed by campylobacteriosis to human health in developing countries where the practices for food handling and hygiene are different from industrialised countries is not clear. In the present study, we aimed to identify the potential zoonotic sources of *Campylobacter* associated diarrhoea in South India and if there was any species-specific risk.

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Materials and Methods

Stool samples were collected from children with diarrhoea aged less than 5 years, admitted to the institution between January 2003 and May 2006 for studies on diarrhoeal etiology. Written informed consent was obtained prior to enrolment from parents or guardians of the children. The study was approved by the institutional review board. Diarrhoea was defined as the passage of three watery stools in a 24-h period. Diarrheal samples from animals were collected from a veterinary clinic and several dairy farms near Vellore between February 2007 and May 2008. Faecal samples from 25 chicken from a poultry farm in Vellore were also collected in 2009.

Animal and poultry stool samples were treated with proteinase K (2 µg/ml in 20 mM Tris, pH 7.5, 10 mM EDTA, and 0.1% SDS) followed by a previously validated extraction protocol with alkaline (1M KOH and dithithreitol), acid (25% HCl and 2M Tris HCl) and phenol chloroform treatment and Qiamp DNA stool minikit extraction (Qiagen, Valencia, CA, USA). DNA extraction of stool samples from children was carried out with the same kit but without pre-treatment of samples. *Campylobacter* spp. PCRs targeting the 16S rRNA gene[2] [Figure 1a] for screening *C. jejuni* and *C. coli* were carried out on all samples. Species identification was carried out on positive samples with *hippuricase* PCR [Figure 1b] (hippuricase gene seen exclusively in *C. jejuni*)[3] and *asp* PCR [Figure 1c] (aspartokinase gene seen in *C.coli* but not in *C. jejuni* and *C. lari*)[4] using previously published primers. Diarrhoeal samples from children were also screened for rotavirus by ELISA (Rotavirus IDEIA, UK), *Cryptosporidium* spp. by microscopy (modified acid fast stain) followed by *SSU rRNA* PCR-RFLP for species determination and diarrhoeagenic *E. coli* by multiplex PCR using previously described methods.[5]

Table 1: *Campylobacter* species and co-pathogens identified in children with diarrhoea

Sample number	Species specific PCR		*Campylobacter* species	Co-pathogens
	hippuricase PCR	asp PCR		
RV210		+	*C. coli*	ETEC, Rotavirus
RV 232	+		*C. jejuni*	EAEC, Rotavirus
RV 283	+	+	*C. jejuni/C. coli*	EPEC, EAEC, Rotavirus
RV 313	+		*C. jejuni*	*Cryptosporidium hominis*
RV 330	+		*C. jejuni*	Rotavirus
RV 331	+		*C. jejuni*	EPEC, *Cryptosporidium hominis*
RV 346	+		*C. jejuni*	EHEC, EAEC, Rotavirus
ICRV 52	+		*C. jejuni*	EAEC, Rotavirus
ICRV 53	+		*C.jejuni*	-
ICRV 56	+	+	*C. coli/C. jejuni*	ETEC, Rotavirus
ICRV 63	+	+	*C. jejuni/C. coli*	Rotavirus
ICRV 87	+		*C. jejuni*	EAEC, Rotavirus
ICRV 89	+		*C. jejuni*	Rotavirus
ICRV 92	+		*C. jejuni*	EPEC, Rotavirus
ICRV 94	+		*C. jejuni*	-
ICRV 121	+		*C. jejuni*	Rotavirus
ICRV 122		+	*C. coli*	-
ICRV 181	+		*C. jejuni*	EAEC, Rotavirus

EAEC – Enteroaggregative *E. coli*, EHEC – Enterohaemorhagic *E. coli*, EPEC – Enteropathogenic *E. coli* and ETEC – Enterotoxigenic *E. coli*

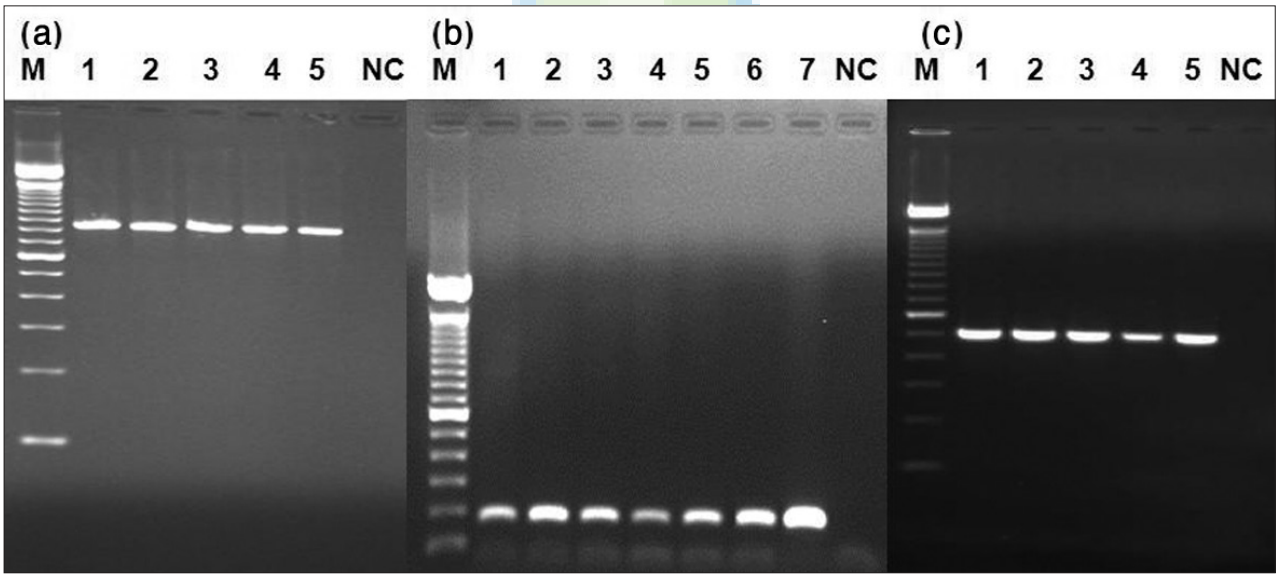

Figure 1: Agarose gel electrophoresis of PCR products for identification of (a) *Campylobacter* spp. (16S rRNA PCR, 820 bp) (b) *C. jejuni* (*hippuricase* PCR, ~180 bp) (c) *C. coli* (*asp* PCR, 500bp). Lane 1 in all gels is a 100 bp molecular weight marker and NC is the negative control

Results

A total of 394 stool samples from children aged less than five years presenting with diarrhoea were screened. The median (inter-quartile range, IQR) age of the children enrolled in the study was 10 months (7) and the mean duration of diarrhoea was 2.8 days. *Campylobacter* spp. were detected in 18 (4.5%) children, a majority of whom (15/18) were co-infected with other pathogens [Table 1]. There were no significant differences in features of diarrhoea between children with *Campylobacter*-associated mixed infections and other children enrolled in this study (data not shown). Of the 18 *Campylobacter* spp. positive samples, 13 children were infected with *C. jejuni* alone, two with *C. coli* alone and three with both *C. jejuni* and *C. coli*.

A total of 627 samples from animals with diarrhoea were collected, including 589 cows (25 were calves), 2 buffaloes, 11 bullocks and 25 goats (11 were kids). The mean duration of diarrhoea was 4.5 days for adult animals, 4 days for calves and 3 days for goat kids. None of the animals were found to be infected with *Campylobacter* spp. When stool from 25 chickens at a poultry farm in Vellore were screened, 16 chickens were found to be infected with *Campylobacter* spp. all of which were characterised as *C. jejuni*.

Discussion

Previous studies from India using culture as a screening tool have documented a wide variation of prevalence rates in children with diarrhoea ranging from 3.2% in Karnataka,[6] 8% from Chennai[7] to 13% from Lucknow[8] and up to 11.1% in asymptomatic controls.[9] In studies using PCR-based methods, *Campylobacter* spp. was found to be associated with 5.7% of diarrhoea in children in South India[5] and 5.1% in North Indian children.[10] In this study, a comparable prevalence rate of 4.5% was seen but *Campylobacter* strains were rarely identified as single pathogens indicating that the role of this organism as the etiological agent of diarrhoea may be questionable. Studies from India and other developing countries have also identified high rates of mixed or polymicrobial infections involving *Campylobacter*.[11-13] However, a recent study in north Indian children documented a higher risk of GBS among children with a history of *Campylobacter*-related diarrhoea[14] demonstrating the importance of detection and prevention of these infections.

Our data indicated that poultry was the major reservoir of infection in this region. Data on exposure to animals or poultry was not available for the children enrolled in the study; therefore, further risk assessment could not be carried out. Previous studies from India have, however, documented poultry as a reservoir of *Campylobacter* spp. with reported prevalence rates of 39.3% and 48%, respectively.[15,16] Our findings of only *C. jejuni* in poultry is also in agreement with data from other parts of India which showed that *C. jejuni* were more frequent than *C. coli* in poultry.[16]

Although the current study did not detect *Campylobacter* spp. in cattle and other animals, studies from India have identified cattle and other livestock as reservoirs for this bacterium.[16] The lack of detection of *Campylobacter* isolates in cattle in our study could either be due to a low burden among these animals or due to the presence of inhibitors affecting PCR on animal faeces.[17,18] Additional techniques, including culture on enrichment and/or semi-selective media or on template DNA obtained from enrichment medium may have improved detection rates.

In conclusion, the present study has shown that *Campylobacter* associated with diarrhoea in south Indian children usually occurs as a polymicrobial infection. Future community-based studies are required for insights into the role of *Campylobacter* in diarrhoea in Indian children. While other studies have reported cattle as a major reservoir of *Campylobacter,* we were only able to identify poultry as a potential source of infection. In addition, the observation that chicken-isolated species were common among human isolates is consistent with other studies where typing has indicated that strains from chickens are often linked to human campylobacteriosis and is an important first step in planning preventive strategies.

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