SEASONALITY OF CAMPYLOBACTER JEUNI ISOLATED FROM RAW MILK

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ABSTRACT

This study was contemplated to investigate the season wise prevalence of Campylobacter jejuni from a total of 3099 raw milk samples collected aseptically from different local vendors, retail outlets and hotels from various zones of Chennai. A total of 42 C. jejuni isolates were obtained with a prevalence level of 1.36%. Seasonal variation in the prevalence of C. jejuni in milk is observed, with a peak in hot weather season (57.1%) followed by south west monsoon season (26.2%) and lowest in cold weather season (4.8%). On statistical analysis, Chi-Square test indicated that the prevalence of C. jejuni was correlated with season i.e. highly significant (P < 0.01). The higher prevalence of C. jejuni in hot weather season is probably attributable to the availability of conducive optimal growth requirements of the organisms and hygienic measures practised in the farm environment and transport of milk.

Key words: C. Jejuni, Milk, Seasonal variation.

INTRODUCTION

Campylobacter was first recognized as a cause of human food borne disease in 1977, and has since become one of the leading sources of acute gastroenteritis in industrialized nations, including the United States (Tauxe 1992). Campylobacteriosis is an acute gastrointestinal infection with severe abdominal pain, fever, nausea, headache, muscle pain, and diarrhea. The length of the incubation period is 3–5 days with symptoms lasting 5–7 days. Infections are typically self-limiting. Campylobacter accounts for 17.3% of all the food related bacterial infections that are reported (Mead et al., 1999). Most (85-95%) human campylobacteriosis cases are due to C. jejuni, with C. coli accounting for the majority of the remainder (5-15%) (Friedman et al., 2000).

The bovine intestinal tract is a known reservoir of C. jejuni and is excreted through feces and animal secretions and dairy cattle get infected through ingestion of water and feeds contaminated with manure. C. jejuni can cause mastitis in cows and it can be shed in milk of carrier asymptomatic cows.

Humans get infected through ingestion of untreated water, contaminated non-pasteurized milk, improperly pasteurized milk (Evans et al., 1996) and poultry meat.(Osano and Arimi, 1999). The first reported instance of human Campylobacter enteritis implicated raw milk as the vehicle for the infective agent was by Levy in U.S.A(1946). The incidence of C. jejuni diarrhoea reported from India is 4-5%. (Nath et al., 1993). Contamination of milk with faeces containing Campylobacter spp. is the primary cause, as Campylobacter mastitis occurs very rarely. Contaminated milk caused some large outbreaks in the past, either at gatherings of people or during farm visits (Evans et al., 1996, Southern et al., 1990, Potter et al., 1983). In Austria, an outbreak of C. jejuni in a youth centre was traced down to the consumption of contaminated unpasteurised milk (Lehner et al., 2000). Direct milk excretion of C. jejuni/coli by clinically healthy cows has been described and implicated in the etiology of human enteritis following consumption of contaminated milk (Orr et al., 1995).

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The incidence of *C. jejuni* in cattle may be seasonal, with peak shedding occurring in either the winter or the summer (Robinson, 1982; Blaser et al., 1983 and Stanley et al., 1998). In Wales, consumption or handling of milk contaminated by birds (magpies and jackdaws) picking at milk bottles was associated with Campylobacter infection during a spring incidence rise (Southern et al., 1990). A bimodal trend with fecal shedding occurring in spring and autumn has also been observed (Stanley et al., 1998). Human campylobacteriosis outbreaks associated with consumption of contaminated milk or water occur in the fall and spring (Skirrow and Blaser, 1992). This seasonal trend may reflect peaks in either fecal shedding in the bovine reservoir or exposure to a common source of contamination (Stanley et al., 1998 and Tauxe, 1992).

In the present study, an investigation has been carried out to find out the seasonal prevalence of *C. jejuni* in raw milk samples collected from different local vendors, retail outlets and hotels from various zones of Chennai so as to chart out a comprehensive profile of the prevalence of *C. jejuni*.

**MATERIALS AND METHODS**

**Milk Samples:** A total of 3099 samples of raw milk were collected aseptically from Madras Veterinary College (MVC) model dairy plant, MVC clinics, different local vendors, retail outlets and hotels from various zones of Chennai so as to chart out a comprehensive profile of the prevalence of *C. jejuni*.

**Isolation of Campylobacter jejuni:** Raw milk samples (usually 50 ml aliquots) were concentrated by centrifugation in a refrigerated centrifuge at 10,000 rpm for 10 minutes (FDA, BAM 1998). The pellet is re-suspended in 10 ml of Brain Heart Infusion broth (FDA-BAM, 1998), pre-enriched microaerobically at 37°C (Humphrey and Muskat, 1989) to facilitate resuscitation process to overcome damage to cells caused by drying, heating, starvation, freezing and/or oxygen radicals and then transferred to 42°C for 24 h in microaerophilic environment. Following pre-enrichment, an aliquot of growth from enrichment tubes is sub-cultured to Campylobacter cefex agar base enriched with 5% defibrinated blood and added antibiotics and incubated for 48 h at 42°C in microaerophilic atmosphere containing 5% (v/v) O₂, 10% (v/v) CO₂ and 85% (v/v) N₂. After 48 hours, plates were examined for the presence of presumptive Campylobacter species colonies. If no growth was observed, plates were incubated for an additional 24 hours and were re-examined and discarded at 72 hours, if negative. Individual non-hemolytic, white to grey colonies suspected of being Campylobacters were selected from the primary culture and were sub cultured onto Campylobacter cefex agar base supplemented with antibiotics and enriched with 5% defibrinated blood and incubated at 42°C for 48 hours with 5% CO₂. Colonies presumptive of Campylobacter were examined microscopically by dark-field illumination for characteristic cellular morphology and darting, twirling motility patterns and also by grams staining and then subjected to various biochemical tests like oxidase, catalase, urease, hydrogen sulphide, reduction of nitrates to nitrites and hippurate hydrolysis test. Biochemically positive isolates were also confirmed genotypically by employing genus specific cadF PCR (Nayak et al. 2005) and species specific hipO PCR (On and Jordan, 2003).

**RESULTS AND DISCUSSION**

All the 44 culture positive samples were also positive by biochemical tests like catalase test, oxidase test and nitrate reduction test. Based on the results of above phenotypic tests suspected isolates were categorised as Campylobacter spp. as per Cowan and Steel (1993).

Based on hippurate hydrolysis test, 42 out of 44 were identified as *C. jejuni* and the remaining two as other Campylobacter spp. All the 44 culture and biochemical positive isolates were subjected to genus specific cadF gene PCR, which is meant for adhesion of Campylobacter to the erythrocyte fibronectin and expected product size 400 bp corresponding to cadF gene was obtained and thus confirmed as Campylobacter spp. which yielded a cumulative prevalence of 1.42%.

Similarly all the 44 isolates were analysed for species specific hipO gene PCR and 42 out of 44 yielded an expected size of 589 bp fragment and
found positive for *C. jejuni*, which gave a prevalence of 1.36%. The remaining two hippurate negative isolates were of *C. coli*. Reference strain obtained from the Department of Microbiology, Institute of Cholera and Enteric Diseases, Calcutta and used as positive control.

The observation of seasonal variation in the present study for the prevalence of *C. jejuni* in milk (Table 1), with a peak in hot weather season (57.1%) followed by south west monsoon season (26.2%) during which time also hot weather prevails to some extend and lowest in north east monsoon season (winter) concurs to the earlier findings of peak in summer and distinctly reduced prevalence of *C. jejuni* in winter as reported by Robinson (1982). This is supported by a study of Bhadra *et al.* (1992) who examined 857 children (aged between 1 day and 60 months) admitted to National Institute of Cholera and Enteric Diseases, Calcutta and reported that strains of *C. jejuni/coli* were isolated throughout the year with higher isolation rates during the summer and monsoon months. Higher summer prevalence of *Campylobacter* spp. in dairy cattle has been reported by others as well (Meanger and Marshall, 1988). It is also supported by the seasonal variation in *Campylobacter* spp. in broilers, with a peak in the summer, as observed by Berndtson *et al.*(1996).

A striking feature of campylobacteriosis in temperate countries is the seasonal variation, with one or two incidence peaks occurring in spring, summer or early autumn (Nylen *et al.*, 2002; Kovats *et al.*, 2005). The seasonal variations in nine European countries show a remarkably consistent pattern from year to year (Nylen *et al.*, 2002). The seasonality pattern is still largely unexplained, although it has been shown to be related to climatic factors. Higher maximum or average temperatures, especially in combination with many hours of sunlight, may be associated with higher campylobacteriosis incidence (Patrick *et al.*, 2004; Louis *et al.*, 2005). Seasonal peaks in *Campylobacter* prevalence in broilers and other potential sources have been suggested to be related to the seasonal variations in humans. Ekdahl *et al.*(2005) Nichols (2005) attributed increase in the population of flies as important vectors for infection transmission during the summer. Another suggested cause of the seasonal peaks is human behaviour that may be more common during the warmer season, such as barbecuing, camping, swimming in lakes and rivers, and drinking water from streams and lakes (Nylen *et al.*, 2002).

A seasonal variation in campylobacter in broilers, with a peak in the summer, was observed by Berndtson *et al.*, (1996). It seems that it is difficult to control campylobacter in broilers in the summer. This seasonal variation has also been observed in broilers in many other countries such as Norway (Hofshagen and Kruse, 2005), Denmark (Wedderkopp *et al.*, 2000 and Bang *et al.*, 2003), the Netherlands (Bouwknegt *et al.*, 2004), and in the number of human cases of campylobacteriosis both in Sweden and in other temperate countries (Nylen *et al.*, 2002; Patrick *et al.*, 2004; Kovats *et al.*, 2005 and Meldrum *et al.*, 2005).

The present findings is also supported by an earlier study in Tamil Nadu in broilers which revealed that prevalence of *Campylobacter* spp. in broilers during March–June was 94.54%

### TABLE 1. Season wise prevalence of *Campylobacter jejuni* in raw milk.

<table>
<thead>
<tr>
<th>Season</th>
<th>No. of samples</th>
<th>Isolates obtained zone wise</th>
<th>Total</th>
<th>% of Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>North</td>
<td>South</td>
<td>West</td>
</tr>
<tr>
<td>CWS</td>
<td>785</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>HWS</td>
<td>814</td>
<td>8</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>SWM</td>
<td>720</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>NEW</td>
<td>780</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>3099</td>
<td>15</td>
<td>7</td>
<td>11</td>
</tr>
</tbody>
</table>

\[\chi^2 = 25.70^{**}\]

CWS : Cold weather season (December – February)
HWS : Hot weather season (March – May)
SWM : South west monsoon (June - August)
NEM : North east monsoon (September - November)
This seasonal variation has also been observed in broilers in many other countries such as Norway (Hofshagen and Kruse, 2005) and was also supported by the recent reports of *Campylobacter* spp. in broilers in Sweden (Hansson *et al.*, 2007).

**CONCLUSION**

Seasonal variation in the isolation rate of *Campylobacter* from raw milk samples was observed to be maximum in hot weather season in the months of March, April and early May and dropped in North East Monsoon season in the months of December, January and February, the cold weather season. Variation in the prevalence of *Campylobacter* isolates from milk samples and other food commodities reported in other studies may be a result of different sampling techniques employed, seasonality and laboratory methodologies employed and may also be due to the reason that the studies were carried out in different countries at different times.

**REFERENCES**


