Introduction

Campylobacter infection, especially due to Campylobacter jejuni, is one of the most widespread infectious diseases in both developed as well as developing countries (Kaakoush et al., 2015). The thermophilic campylobacters, mainly C. jejuni and C. coli, account for nearly 400-500 million cases each year worldwide (Ruiz-Palacios, 2007). Campylobacter spp. are widely distributed among food animals, and their presence as commensals in the intestinal tracts of these animals complicates the scenario. The ingestion of faecal contaminated chicken meat or poor food handling practices associated with raw chicken represents the primary route of transmission to humans (OIE Terrestrial Manual, 2008). It has been estimated that chicken as a reservoir might account for between 50% and 80% of the cases (EFSA, 2010). Besides poultry, raw milk, dairy products, cattle, pigs, untreated water, and sewage have been reported as sources for Campylobacter spp. A detailed understanding of the epidemiology of human infections and animal incidence is essential for the control of campylobacteriosis.

Thermophilic campylobacters cause a spectrum of diseases in man ranging from mild, self-limiting non-inflammatory to severe inflammatory bloody diarrhea with pyrexia, abdominal cramps and bacteraemia. The infection is self-limiting, but in a fraction of the patients, serious post-infection complications like Guillain-Barre Syndrome (GBS) and Miller Fisher Syndrome occur. Other complications including intestinal hemorrhages, mesenteric adenitis, toxic megacolon, hemolytic uremic syndrome, reactive arthritis and rarely, meningitis have also been reported.

Sporadic studies conducted in different parts of India have revealed presence of Campylobacter spp. in various species of animals and birds, foods of animal origin, raw milk samples, and foods of plant origin. Common biotypes and serotypes have been isolated from human cases and birds. The occurrence of this organism has been documented from cases of human diarrhea, GBS and from animal handlers. However, the figures for actual prevalence and economic losses due to campylobacteriosis in India are unfortunately not available. Moreover, the lack of knowledge among the
public regarding zoonotic infections and in particular about campylobacteriosis is a matter of concern. This reinforces an immediate need for systematic epidemiological study on Campylobacter infections in this part of the world. The present study was carried out to investigate the occurrence of thermophilic Campylobacter spp. from diverse sources.

Materials and Methods

Sample collection and isolation

A total of 490 samples comprising chicken caecum (n=95), quail caecum (50), chicken meat and skin (95), milk (100), dog faeces (50), pig faeces (50) and human stools (50) were screened for the presence of Campylobacter spp. The caecal samples were subjected to direct plating on modified charcoal cefoperazone deoxycholate agar (mCCDA) supplemented with CCDA selective supplement (FD135; HiMedia, India). The swabs were moistened and streaked directly on to the agar and incubated under appropriate conditions. Similarly, milk, dog and pig faecal samples (BB) (Titan, India) and incubated under microaerophilic atmosphere (10% CO2, 5% O2 and 85% N2) at 42°C for 48 h. The chicken meat samples (10 g each) were enriched in 90 ml Bolton broth (BB) (Titan, India) and incubated under appropriate conditions. Similarly, milk, dog and pig faecal samples and human stool samples were enriched in 1:10 ratio in BB. A loopful of the contents from enriched samples was streaked onto mCCDA and incubated under microaerophilic conditions at 42°C for 48 h.

The presumptive colonies were picked from each mCCDA plate and transferred to 7% blood agar (Columbia blood agar base, Oxoid) for isolation of pure colonies. The isolates were subject to Gram staining, oxidase and catalase tests, aerobic growth test and glucose utilization test. The preliminary speciation was done by subjecting the isolates to hippurate hydrolysis and sensitivity to nalidixic acid (30 µg) and cephalothin (30 µg) to classify isolates as C. jejuni, C. coli, which did not show any amplification. A multiplex PCR assay was used for the characterization of genus confirmed isolates. The reaction mixture (25 µl) comprised 2.5 µl of 10x Dream Taq Buffer, 2.5 µl of 2 mM of each dNTP, 15 pmol of each primer, 1 U Taq polymerase, 3 µl of bacterial DNA and nuclease-free water to make up to 25 µl. The cycling conditions followed were: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 1 min. Final extension was done at 72°C for 7 min.

The PCR products (10 µl aliquots) were analyzed by electrophoresis on 1.5% agarose gel (Gonite, USA) at 90 V for 45 min, with 0.5x TBE as running buffer. DNA bands were stained with ethidium bromide (0.5 µg/ml) and gel pictures documented using Alpha Imager gel doc unit (Alpha Innotech Corp., USA). The band positions were determined by comparing with GeneRuler 100 bp Plus DNA ladder (Fermentas).

Results and Discussion

A total of 46 isolates including 33 C. jejuni and 13 C. coli were isolated from all the collected samples. Out of the 46 isolates, 33 were found positive for hippurate hydrolysis and were classified as C. jejuni. All the isolates were sensitive to nalidixic acid and resistant to cephalothin.

The conserved 16S rRNA gene was targeted for species confirmation and all the 46 isolates yielded an expected amplicon of 816 bp (Fig. 1). The specificity of the primer set used was deduced by checking it against DNA isolated from Salmonella, Arcobacter spp. and E. coli, which did not show any amplification. A multiplex PCR assay was employed for identification/confirmation of 4 species of thermophilic campylobacters viz., C. jejuni, C. coli, C. lari and C. upsaliensis. The lipid gene, lpxA was targeted in this assay and yielded discriminatory band sizes for speication. Of the 46 isolates obtained in this study, 33 yielded amplicon of 331 bp for C. jejuni and the rest 13 gave an amplicon of 391 bp for C. coli (Fig. 2). However, the other two species targeted in the present study, C. lari and C. upsaliensis were not detected in any of the samples. PCR seems to be a rapid alternative to differentiate between the species in place of the time consuming and laborious biochemical tests. Also, reports
exist regarding hippurase negative C. jejuni, which renders the hippurase test a non-foolproof test to differentiate C. jejuni species from the rest. However, no hippurase negative C. jejuni isolates were encountered in this study.

Thermophilic campylobacters are major foodborne pathogens of animal origin and a leading cause of bacterial gastroenteritis in humans (Jeon et al., 2010). They are gaining increasing attention of public health workers owing to their complex epidemiological cycle, potential for human health hazard and the economic losses they inflict. The samples from a diverse range of sources were examined in the present study for isolation of Campylobacter spp. Out of 490 samples screened, 46 were found positive for Campylobacter spp. with an overall prevalence rate of 9.39%. Highest isolation rate was observed in chicken and quail caecum (17.8% and 18%, respectively), followed by chicken meat (9.47%), pig faeces (6%), dog faeces (6%), human stools (4%) and milk (3%). A total of 46 thermophilic campylobacters were isolated comprising of 33 C. jejuni (81.25%) and 6 C. coli (18.75%). The findings support the observations of Vandamme (2000) who summarized reports from various countries with the conclusion that approximately 95% of Campylobacter infections are estimated to be caused by C. jejuni with C. coli responsible for only 3-4% cases. The other two important thermophilic campylobacters, C. lari and C. upsaliensis were not detected in the present study.

Out of the 50 human stool samples, only 2 (4%) yielded C. jejuni, which were recovered from children aged between 3 to 5 years. Both positive samples were from males; one had a history of diarrhea for the past day, while another had normal stools but was suffering from pyrexia of unknown origin. Moore et al. (2005) also reported that Campylobacter infection was common in males and the findings are in agreement. Sharma (2004) and Singh (2006) recorded comparatively higher incidence rate of 7.5% and 6.89%, respectively in Bareilly. Rizal (2011) reported an overall prevalence of 3.8% C. jejuni in human stool samples from North-Eastern part of India.

In the present study, the overall prevalence of Campylobacter spp. in poultry intestinal samples was 17.8%, with C. jejuni accounting for 64.7% of the isolates (11.47 isolates). Saleha (2002) also recorded C. jejuni as the most frequently isolated species from poultry than C. coli. Our findings are in agreement with the report of Deckert et al. (2010) who isolated 90% C. jejuni among the total isolates in retail chicken in Canada. Sharma (2004) and Singh (2006) reported 21.8% and 14.28% isolation rates, respectively. Rajagunalan et al. (2014) reported 15.89% occurrence in caecal and faecal samples of chicken.
The quail caecum samples examined in the study revealed incidence rate of 18% with 88% *C. jejuni* isolates. Abdulazeez and Thompson (2006) reported 8 isolates (2.2%) from quail caecum from Turkey. In a study by Rahimi and Tajbakhsh (2008) in Iran, highest prevalence was recorded in quail meat (68.4%) in comparison to chicken, turkey and ostrich meats. This emphasizes the fact that quails are also potential reservoirs of *Campylobacter* spp. similar to chicken.

Analysis of chicken meat samples for thermophilic campylobacters showed an incidence rate of 9.47%. Our findings support the reports of various studies conducted in different parts of India. Barua (2003) reported 12% and Sharma (2004) reported 13.9% contamination rates in samples collected in and around Bareilly region. Rajkumar et al. (2010) found a prevalence of 18% from unorganized and 12% from organized farms from chicken skin samples in Uttar Pradesh. The incidence rate in dog faeces in the present study was 6% with 2 isolates of *C. jejuni* (66.6%) and one isolate of *C. coli* (33.3%). Similar rate of incidence has been reported by Chattopadhyay et al. (2001), who isolated 4 *C. jejuni* from healthy and diarrheic dogs with an overall incidence of 13.3% from Kolkata. The pig faecal samples showed an isolation rate of 6%. Although, pigs are considered one of the main reservoirs of *C. coli*, only one isolate was found to belong to this species among a total of 3 isolates. In contrary, Sharma (2004) reported a prevalence rate of 17.5%. Chattopadhyay et al. (2001) reported prevalence of 37.1% in healthy pigs. Raw milk samples are among the potentially important sources of *Campylobacter* spp. A total of 100 raw milk samples were screened and revealed a prevalence of 3%. In a study conducted in Chennai, Elango et al. (2009) reported a prevalence of 1.42%. Hudson et al. (1999) reported <1% prevalence in milk samples. However, many researchers have emphasized the fact that pasteurization kills this bacteria.

In conclusion, high prevalence rates of thermophilic campylobacters were observed in chicken and quail caecum, followed by chicken meat, pig faeces, dog faeces, human stools and milk. The high prevalence rate in quails emphasizes the fact that they are potential reservoirs of *Campylobacter* spp. similar to chicken. Further, prevalence in dogs highlights the role of pets in transmission of this disease causing agent. The multiplex PCR assay was found to be a rapid and reliable tool for species identification.

### Acknowledgements

The authors are thankful to the Director, ICAR-Indian Veterinary Research Institute for supporting this research and providing facilities. The present work was carried out under the ICAR funded project ‘Outreach Programme on Zoonotic Diseases’.

### References


Hudson, J.A., C. Nicol, J. Wright, R. Whyte and S.K. Hasell. 1999. Seasonal variation of *Campylobacter* types from human cases,


