

*Original Research***Epidemiological Studies on Animals and Humans as Reservoirs of Thermophilic Campylobacters****R. Pandey, Maansi¹, R. Rautela² and R. N. Trivedi³**

GBPUAT, Pantnagar, Uttarakhand, INDIA

¹Assistant Professor, Department of VPE²M.V.Sc.Student, Department of VPE³PhD student, Department of Microbiology and Immunology**Manuscript is the part of the M.V.Sc. thesis****Corresponding author:** maansi2000@rediffmail.com

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Abstract

A total of 730 samples comprising of poultry caeca (210), poultry meat (111) along with faeces of poultry (180), goat (26), sheep (23), pigs (78), calves (20) and humans (82) were collected from Pantnagar and nearby areas of Uttarakhand. Biochemical and molecular tests confirmed 39 isolates belonging to genus *Campylobacter* with 21 *C. jejuni* and 18 *C. coli* by multiplex PCR. Highest isolation rate was recovered from poultry caeca (7.62%) followed by poultry faeces (7.22%) chicken meat (3.60%), humans stools (3.66%), pigs faeces (2.56%) and calves faeces (5%). No isolate was recovered from sheep and goat samples. Absolute dominance of the *C. jejuni* and *C. coli* was not observed among the samples. Pantnagar and Bareilly regions observed occurrence of thermophilic campylobacters with varying prevalence. The presence of thermophilic campylobacters in animals of the region could be potential source of spread in humans.

Key words: Prevalence, Reservoirs, Thermophilic Campylobacters, Uttarakhand

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Introduction

Among all the food borne organisms associated with enteric infection, *Campylobacter* species is one that affects a wide range of animals and humans. The alimentary tract of animals, particularly domesticated mammals and birds are the principle reservoir of the pathogenic campylobacters. Humans get infected mainly due to contamination of foods such as egg, chicken, pork, milk products,

fruits and vegetables. Poultry meat serves as an important vehicle for food borne transmission of *C. jejuni* (WHO, 2009). The gastrointestinal infection in humans may result in life threatening illness or death. Hence, requires a particular concern. *Campylobacter* spp. responsible for human illness are mainly thermophilic campylobacters preferably *C.jejuni* and *C.coli* (Altekruse *et al.*, 1999) followed by *C. upsaliensis* and *C. lari* (Labarca *et al.*, 2002). Approximately 95% of enteric *Campylobacter* infections in developed world are estimated to be caused by *C. jejuni* and 3-4% by *C. coli* (Vandamme, 2000 and Fitzgerald *et al.*, 2008). Prevalence of these thermophilic campylobacters in wide range of animals and their shedding off may account for gastrointestinal illness in humans. Severe or prolonged infection even warrants hospitalization requiring antimicrobial therapy (Altekruse *et al.*, 1999). Efficient and rapid monitoring of *Campylobacter* in animals is necessary to identify the potential sources and the extent of its occurrence as a probable cause of illness in humans. In view of the above, the present study explores the reservoirs of thermophilic campylobacters in the region.

Materials and Methods

Study Area and Source of Samples

A total of 730 samples comprising of poultry caeca (210), poultry meat (111) along with faeces of poultry (180), goat (26), sheep (23), pigs (78), calves (20) and humans (82) were collected from Pantnagar and nearby areas (Haldwani and Bareilly). All the samples were collected in sterile sample collection vials (Tarsons) and brought in ice packs to the laboratory where they were processed immediately for the isolation of *Campylobacter* spp.

Isolation and Identification

The meat, faecal and caecal samples had to undergo different processing procedures as per protocol (OIE terrestrial manual (2008). All the faecal samples collected from poultry, goat, sheep and pig were enriched in modified charcoal cefoperazone deoxycholate (mCCD) broth under microaerophilic conditions at 42°C for 48 hrs. The enriched culture from the broth was then streaked onto modified charcoal cefaperazone deoxycholate agar (mCCDA) media and incubated at 42°C with 5.2% tension in a CO₂ incubator for 48 hrs.

The caeca brought to the laboratory were cut open using sterile scissor and a loopful of inoculum was streaked directly onto mCCDA plates and incubated under microaerophilic conditions consisting of 5.2% CO₂ at 42°C for 48 hrs. The meat samples of poultry (approximately 10g) were homogenized in approx. 20 ml of phosphate buffer saline (PBS) solution. The prepared suspension was enriched in Bolton broth at 1: 9 ratio and incubated under microaerophilic condition at 42°C for 48 hrs. A loopful of broth was then streaked on mCCDA plates with 5% tension in CO₂ incubator for 48 hrs.

Phenotypic *Campylobacter* species identification method included typical appearance of colonies (1-2 mm size, circular, flat to slightly raised, sticky, spreading and shiny grey) on mCCDA plates), a distinctive microscopic feature when gram stained (spiral or S-shaped) and biochemical tests such as catalase, oxidase, nitrate reduction, urease and H₂S production on TSI (Lastovica and Allos, 2008). All the presumptive *Campylobacter* isolates that were catalase, oxidase, nitrate reduction and indoxyl acetate hydrolysis positive while urease and TSI negative were subjected to DNA isolation. All the isolates were stored in 15-20% glycerol solution at 80°C till further use.

Molecular Confirmation using Multiplex PCR

The bacterial genomic DNA of putative *Campylobacter* isolates were extracted using Hi-Pura genomic DNA extraction kit (Hi-media) as per the directions of manufacturer. The primer set used (*cadF*, *hipO* and *asp*) along with product size and specificity is listed in Table 1.

Table 1: Primers used in mPCR reaction to identify genus and species of *Campylobacter*

Primer Name	Primer Sequence (5'-3')	Target Gene	Product Size	Primer specific for	Reference
<i>Cad F</i>	F- TTGAAGGTAATTTAGATATG R- CTAATACCTAAAGTTGAAAC	<i>cadF</i>	400bp	Genus <i>Campylobacter</i>	Nayak <i>et al.</i> (2005)
<i>HIP400</i> <i>HIP1134</i>	F- GAAGAGGGTTTGGGTGGTG R- AGCTAGCTTCGCATATAACTTG	<i>hipO</i>	735bp	<i>C. jejuni</i>	Linton <i>et al.</i> (1997)
<i>CC18</i> <i>CC519</i>	F- GGTATGATTTCTACAAAGCGAG R- ATAAAAGATATCGTCGCGCGTG	<i>asp</i>	500bp	<i>C. coli</i>	Linton <i>et al.</i> (1997)

The multiplex PCR (mPCR) was set up in a 25 µl reaction mixture with 2.5 µl of 10X PCR assay buffer, 1 µl of 10mM dNTP mix, 1µl of 25pmol / µl of each primer set, 0.25 of 5 Unit / µl Taq DNA polymerase, 5 µl of 50 ng /µl T Genomic DNA with q.s.25 µl sterile distilled water. The initial denaturation was performed at 95°C, 15min, 30 cycles of denaturation(94°C,1min) ,annealing (50.2°C,1 min) and extension (72°C, 1min). Final extension was performed at 72°C, 7 min. The PCR product was analyzed by electrophoresis in 1.5% agarose gel using gel documentation system.

Results and Discussion

Thermophilic campylobacters isolated on mCCDA plates after incubating under microaerophilic condition at 42°C for 48 hrs revealed characteristic small (1-2mm) colonies which were flat to slightly raised, greyish and moistened with spreading tendency. Biochemical identification detected fifty six isolates as catalase, oxidase and nitrate reduction positive while urease and H₂S negative. On Gram's staining, the colonies appeared as gram negative with typical 'S' shaped rods or spiral rods. Out of 56 presumptive isolates, only 39 isolates produced an amplicon of 400bp (*Campylobacter* genus

confirmation) through multiplex PCR (Fig). Of these, 21 produced an amplicon size of 735bp (*hipO*) confirming them as *C. jejuni* and 18 isolates amplified at 500bp, confirming as *C.coli* (*asp* gene).

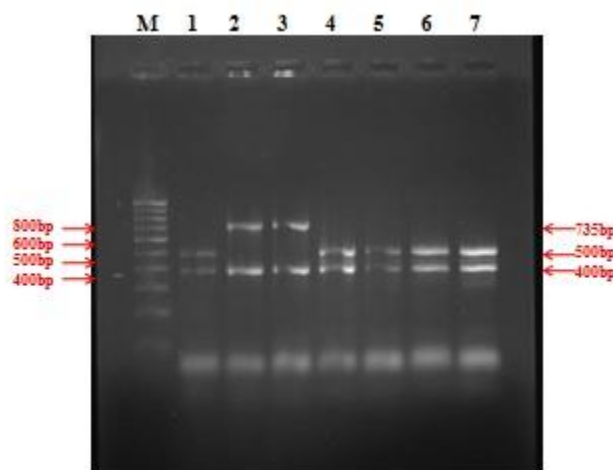


Fig: Multiplex PCR showing amplified products of *Campylobacter*
Lane M: 100bp ladder; Lane 1-7: Genus *Campylobacter*(400bp),
Lane 1,4,5,6,7: *C.coli* (500bp) and Lane 2,3: *C.jejuni* (735bp)

Species Wise Prevalence

The 39 samples detected positive for *Campylobacter* spp. revealed an overall prevalence of 5.34%. The findings were concurrent to Rajagulanan (2010) who accounted 6.9% prevalence rate of *Campylobacter* spp. in the study area whereas Prasanna (2013), Pant (2011) and Monica (2014) observed an overall prevalence rate of *Campylobacters* as 11.66%, 16% and 7.36% respectively in the same area. Out of total of 39 thermophilic *Campylobacter* detected, 21 (53.84%) belonged to *C. jejuni* and 18 (46.16%) were detected as *C. coli*. Total prevalence in the area showed variation which may be attributed to the difference in the season of collection of samples or the health status of the animals. Komba *et al.*, 2103 has reported investigations that associate season with the colonization of the organisms in the intestine of the animals.

Highest isolation rate was observed from poultry caeca (7.62%) followed by poultry faeces (7.22%) chicken meat (3.60%), humans stools (3.66%), pigs faeces (2.56%) and calves faeces (5%). The observations were in agreement with the findings of Humphrey *et al.*, 2007 who observed that not only the poultry digestive tract but also calves, pigs and human digestive tract act as significant reservoir for *Campylobacter* species. In a study conducted by Workman *et al.* 2005, they also recorded higher prevalence (94.2%) in chicken compared to other species. However, they did not find any isolate from cows. Out of 20 calf samples, only one isolate was confirmed as *C. coli*. Cattle have been reported to be the reservoir of campylobacters by many workers (Stanley and Jones, 2003; Hakkinen *et al.*, 2007 and Heriberto, F. and Marianne, H. 2009). This species significantly disseminates *Campylobacter* sub-types

that can cause disease in the human community. The results obtained during the study showed an isolation rate of 5% in calves. The samples mostly belonged to the ill and diarrhoeic animals. Hald *et al.*, 2016 also reported prevalence of campylobacters in livestock with wild birds being the source of infection. The faecal examination of pigs revealed only 2(2.56%) isolates out of 78 samples. Pigs are reported as a reservoir for thermophilic campylobacters and a high prevalence (90.5%) of these organisms was recorded by Workman *et al.*, 2005. All the isolates were confirmed as *C.coli* which was in contrast to our study where all the recovered isolates from pigs were *C. jejuni* (Table 2). Had the number of samples been more in our study, a better estimation of the prevalence in pigs could have been done.

Table 2: Species distribution of *Campylobacter* isolates

S. No.	Sample Source	No. of Isolates	<i>C. jejuni</i>	<i>C. coli</i>
1.	Poultry caeca	16 (7.62%)	7 (43.75%)	9 (56.25%)
2.	Chicken meat	4 (3.60%)	3 (75%)	1 (25%)
3.	Poultry faeces	13 (7.22%)	7 (53.85%)	6(46.13%)
4.	Pigs faeces	2 (2.56%)	2 (100%)	0 (0.0%)
5.	Sheep and goats	0 (0.0%)	0 (0%)	(0.0%)
6.	Calves faeces	1 (5%)	0 (0%)	1(100.0%)
7.	Human stools	3 (3.66%)	2 (66.67%)	1 (33.33%)
	Total	39 (5.34%)	21 (53.85%)	18 (46.15%)

None of the isolates were recovered from sheep and goats. A study conducted by Zweifel and Stephen, (2004) also concluded low prevalence of campylobacters in sheep and goats. However, on the other hand, Lazou *et al.* (2014) reported 30% prevalence in sheep and goat intestinal samples. Rapp and Ross, 2012 found the presence of campylobacter organisms in a goat farm and attributed its presence to the unhygienic conditions prevailing in the farm, one of the risk factors associated with the human or animal illness. In our study, the absence of isolates among sheep and goat may be either due to less number of samples and/or better standards of hygiene at farm. Occurrence of campylobacters (3.66%) was also observed in human stool samples, out of which 2(66.67%) were *C.jejuni* and 1 (33.33%) *C. coli*. The pathogen is of high public health significance and is amongst the important food borne illness. Reports of human infection with poultry as a source is well documented (Oporto *et al.*, 2007; Khoshbakht *et al.*, 2016). Both *C.jejuni* and *C. coli* species were identified among the obtained isolates without the prominence of a single species unlike many studies which have reported either *C. jejuni* or *C. coli* having species prevalence (OIE terrestrial manual, 2017; Varela *et al.*, 2007) to be dominant among the two.

Prevalence as per Location

Two locations (Pantnagar and Bareilly) were selected for the poultry caeca, chicken meat and poultry droppings (Table 3). Samples of other species as source were not studied for location difference as they

were collected from a single location. Human samples collected belonged to Haldwani (40), Rudrapur (28) and Pantnagar (14) regions.

Table 3: Location wise number of samples of poultry

Location	Sample Source		
	Poultry caeca	Chicken meat	Poultry droppings
Pantnagar	137(6.57%)*	68(4.41%)	136(5.15%)
Bareilly	73(9.59%)	43(2.33%)	44(13.64%)
Total	210(7.62%)	111(3.61%)	180(7.22%)

*Figures in parenthesis denotes prevalence

The 210 poultry caeca samples collected from Bareilly (73) and Pantnagar (137) regions showed more prevalence in Bareilly (9.59%) than Pantnagar (6.57%). Pantnagar recorded more *C.jejuni* than *C. coli* whereas in Bareilly, *C. coli* isolates were more prevalent than *C. jejuni*. The findings are in agreement with Prasanna (2013) who also recorded higher prevalence of *C. jejuni* in Pantnagar. A study conducted by Malik *et al.* (2014) in Bareilly, also showed more *C. coli* than *C. jejuni* as in our study. The difference in occurrence of campylobacters in Pantnagar and Bareilly may be due to difference in breeding and feeding practices of poultry of the areas. However, analysis of findings of current as well as prior study revealed that there is uniform chance of infection of poultry caeca by either species of *Campylobacter*. The random distribution of data indicates that poultry caeca offers similar environmental conditions to both the species of *Campylobacter*.

Out of total campylobacters present in chicken meat (3.61%), the samples collected from Pantnagar retail shops observed more presence (4.41%) than Bareilly (2.33%) region. *C. jejuni* species was more prevalent in both the regions, indicating that the chicken meat offers more favorable conditions to *C. jejuni* species than *C. coli* as also reported by Prasanna (2013) and Monika (2014). The samples of poultry droppings collected from Bareilly recorded higher prevalence (13.64%) than Pantnagar (5.15%). Among Pantnagar poultry dropping samples, the occurrence of *C. jejuni* was more than *C. coli* as also recorded by Saleha (2002) in poultry droppings. However, at Bareilly *C. coli* were found to be more prevalent than *C. jejuni*, similar to the study done by Malik *et al.* (2014) in Bareilly. Human samples (82) collected from Haldwani (40), Rudrapur (28) and Pantnagar (14) revealed 7.14% and 2.5% presence in Rudrapur and Haldwani regions respectively. However, no isolate was recovered from Pantnagar. Out of the total 3 samples isolated, 2 were *C. jejuni* and 1 was confirmed as *C. coli*. This finding was in variance to Rajagulanan (2010) and Prasanna (2013) who could not recover any isolates from Haldwani region but were able to find some isolates from Pantnagar region.

In a prior study by Prasanna (2013), all the isolates of human stool were of *C. jejuni*, whereas in present study both species were present, in agreement with study by Rajagulanan

(2010). Friedman *et al.* (2000) also reported *C. jejuni* to be twelve times more responsible for human campylobacteriosis than *C. coli*. However, Rajagulanan (2010) observed major prevalence of *C. coli* than *C. jejuni* which was in contrast to present findings.

Age and Sex Wise Prevalence in Humans

The data for the age and sex could only be recorded for humans. Human samples were divided into different age groups. Our study revealed that highest rate of 10.5% from the age group (16-31years) followed by 4.6% in the age group of 32-47 years. Higher infection may result due to dietary differences among the age groups, contact with animals or hygienic practices followed by them. The results coincide with earlier findings of Shaheen (2011) and Prasanna (2013) who also found higher prevalence in these two age groups. Chuma *et al.*, 2016 have reported 19% illness in children less than 5 years of age in Tanzania. Rosenquist *et al.*, 2003 analysed people in the age of 18-29 years to be at the highest risk of developing campylobacteriosis. Sex has also been associated as one of the risk factors for campylobacteriosis (Mdegela *et al.*, 2006). Of 82 human samples screened, 38 belonged to females while 44 were from males. All the 3 isolates recovered from humans belonged to males (3/44). The results obtained were collaborative to Prasanna (2013) and Moore *et al.* (2005) who also observed *Campylobacter* infection to be more among males. However, the reports of Rizal *et al.* (2010) concluded more prevalence in females than males.

Conclusion

Human campylobacteriosis is a frequently diagnosed disease. Most infections are caused by thermophilic campylobacters having their reservoirs in the warm blooded animals with poultry being the preferred hosts. The source of infection to humans is attributed to the consumption of contaminated meat or through environmental contamination. Our study revealed the presence of thermophilic campylobacters in the animals and humans of the area with highest prevalence in poultry. With much prevalence in the area under study, risk to humans may arise either through the contaminated environment or through contaminated food chain. In order to prevent human infections, a strict biosecurity measure undertaken at the primary production site holds high significance. Cases of human campylobacteriosis often go unnoticed and misdiagnosed. The study is highly significant in terms of the increasing incidences of food borne illnesses. This work will also help to create a good database of the prevalence in the area.

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