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Screening of beef and raw cow milk from central Kerala for *Campylobacter* spp. using multiplex PCR#

Devika D. Sunil[1], Binsy Mathew[2*], C. Latha[3], Deepa Jolly[2] and K. Radha[4]
Department of Veterinary Public Health
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651
Kerala Veterinary and Animal Sciences University
Kerala, India

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Abstract

Campylobacter spp. is considered one of the leading causes of diarrhoeal disease. Due to the prevalence of this organism in the gut microbiota of all warm-blooded animals, the chance of contamination is more through animal products. The present study was conducted to assess the occurrence of Campylobacter spp. in milk and beef of central Kerala. A total of 200 beef and 200 raw cow milk samples were collected from various retail outlets and milk societies of Thrissur and Ernakulam districts. Campylobacter spp. were isolated by conventional culture technique on Modified charcoal-cefoperazone-deoxycholate agar. A molecular technique targeting the genus-specific 16S rRNA gene further confirmed the culture and biochemically positive isolates. The species-specific mapA and ceuE genes were targeted for C. jejuni and C. coli, respectively. None of the milk samples collected were positive for Campylobacter spp. However, three per cent of beef samples were tested positive for Campylobacter spp.

Keywords: *Campylobacter spp., milk, beef*

Campylobacter spp. is a normal inhabitant in the intestinal tract of common food animals like poultry, pig, cattle, sheep and shellfish. Campylobacteriosis is one of the four key global causes of diarrhoeal diseases (WHO 2020). The disease is mainly transmitted through undercooked meat and meat products especially poultry meat, contaminated with animal faeces and also through contaminated raw milk and drinking water (Jobi, 2016). The affected individuals usually suffer from bloody diarrhoea, stomach pain, cramps, vomiting and fever for three to five days (Zang *et al*., 2018). Arthritis may develop in some individuals as a complication of the disease. Rarely, an autoimmune

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1. *MVSc Scholar*
2. *Assistant Professor*
3. *Director of Academics and Research, KVASU, Pookode*
4. *Associate Professor, Department of Dairy Sciences and Technology*

**Corresponding author: binsymathew@kvasu.ac.in Ph.9072263357*

disease called Guillain-Barre syndrome occurs as a complication to *Campylobacter jejuni* infection in humans, where the immune system damages the myelin sheath of the nerve cells.

The chance of *Campylobacter* contamination in meat during slaughter and milk during its production and handling is high (Hansson *et al*., 2007). So it is very necessary to study the occurrence of *Campylobacter* in milk and meat. Even though the epidemiological data on *Campylobacter* infection in India through milk and beef is limited, it has been reported in other countries (Korsak *et al*., 2014). The present study was carried out to assess the occurrence of *Campylobacter* spp. in beef and raw cow milk in central Kerala.

Materials and methods

Collection of beef and milk samples

The samples were collected from various milk societies and retail meat shops in Thrissur and Ernakulam districts. A total of 400 samples, 200 each of milk and beef, were collected. Approximately 250 mL of milk and 250g of beef samples were collected aseptically, brought to the laboratory under refrigerated conditions and processed for analysis.

Isolation and identification of Campylobacter spp. by culture techniques

The isolation and identification of *Campylobacter* spp. were done by conventional culture technique (OIE, 2017). Milk samples (0.1 mL) were enriched with 9.9 mL of modified charcoal-cefoperazone-deoxycholate (mCCDA) broth and incubated in microaerophilic conditions at 42ºC for 48h. The beef samples (250g) were added to mCCDA and incubated in microaerophilic conditions at 42ºC for 48h. The incubated samples were streaked onto mCCDA agar supplemented with CAT (Cefoperazone, Amphotericin B and Teicoplanin) selective supplement (FD 145), Campylobacter supplement V (FD 067) and Polymyxin B selective supplement (FD 003). It was then incubated under microaerophilic conditions at 42°C for 48 h. Greyish, shiny, flat, moist and mucoid colonies with a tendency to spread were selected for further biochemical tests.

DNA extraction and polymerase chain reaction

The DNA extraction was done by the snap chill method (Swetha *et al*., 2015). A multiplex PCR was standardised to detect the *Campylobacter* spp. *Campylobacter* spp. was detected by targeting genus-specific *16S* rRNA (Vivekanandhan 2022). Species-specific *map*A and *ceu*E genes targeted PCR was used to identify *C. jejuni* and *C. coli* respectively (Athulya 2021). The primers used for identification are listed in table 1. The optimised protocol was carried out with 30µL reaction mixture containing 200 m*M* 10X PCR buffer, 25 *mM* $MgCl_2$, 5 Units of Taq DNA polymerase, 5µL of DNA template and PCR primers. The amplification of genes was carried out with initial denaturation at 95°C for 10 min, followed by 30 cycles of denaturation at 94°C for 1 sec, annealing at 51.8°C for 1 sec and extension at 72°C for 1 min, followed by final extension at 72°C for 10 min. The representative amplicons were purified and outsourced for sequencing.

Table 1. Primers used for identification of *16S* rRNA, *map*A and *ceu*E genes

Primer	Primer sequence	Size (bp)	Reference
16*SrRNA* F	5'GGATGACACTTTTCGGAGC3'	816	Linton *et al*. (1996)
16*SrRNA* R	5'CATTGTAGCACGTGTGTC 3'		
*map*A F	5' CTATTTTATTTTTGAGTGCTTGTG3'	589	Denis *et al*. (1999)
*map*A R	5' GCTTTATTTGCCATTTGTTTTATTA 3'		
*ceu*E F	5' AATTGAAAATTGCTCCAACTATG3'	462	
*ceu*E R	5' TGATTTTATTATTTGTAGCAGCG3'		

Results and discussion

Occurrence of Campylobacter spp. *in milk and beef samples by culture techniques*

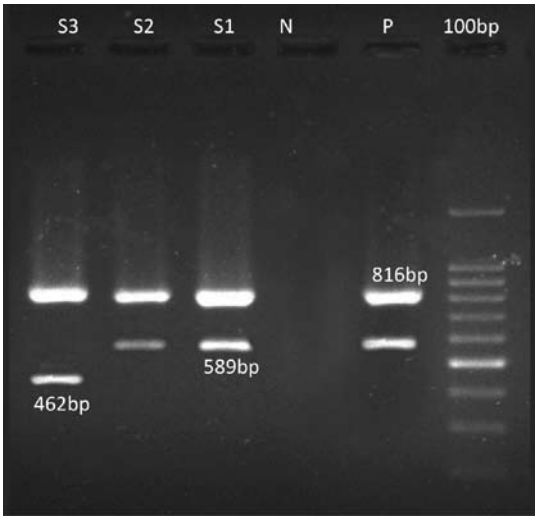
None of the 200 milk samples tested was positive for *Campylobacter* spp. and was in accordance with the findings of Vani (2018), where none of the milk samples (55 samples) were culture-positive. Similar observation was noticed by Wegmuller *et al*. (2003), where out of the 58 raw milk samples screened none were positive for Campylobacter. However, in the study of Modi *et al*. (2015), 2.91 per cent of milk samples collected from different collection points of Anand city, Gujarat, showed the presence of *Campylobacter* spp. Similarly, in the study of Igwaran and Okoh (2020), 37.1 per cent of milk samples collected from Eastern Cape Province, showed the presence of *Campylobacter* spp.

In the case of beef samples tested, *Campylobacter* spp. was isolated from 6 out of 100 beef samples collected from the Ernakulam district. However, none of the samples from the Thrissur district was positive. This result was in accordance with the study of Abiri *et al*. (2014) out of the 200 beef samples collected from Tehran, only 5.5 per cent were positive for Campylobacter. A similar study was conducted by Singh *et al*. (2009) to detect the prevalence of *Campylobacter* spp. in beef in Uttar Pradesh. Out of 300 samples collected, only 5.5 per cent were positive for *Campylobacter*. But, in the study of Bravo *et al*. (2017), none of the beef samples collected were positive for *Campylobacter* spp.

Molecular confirmation of Campylobacter spp.

The six culture and biochemically positive isolates of *Campylobacter* were further analysed by multiplex PCR to detect the genus-specific *16S* rRNA with an amplicon size of 816 bp and species-specific *map*A and *ceu*E genes with an amplicon size of 586 bp and 462 bp respectively (Fig.1). Among the six isolates, five were positive for *map*A and one positive for *ceu*E gene, indicating the presence of 83.33 per cent of *C. jejuni* and 16.66 per cent of *C. coli*. The amplicons obtained were sequenced in a commercial sequencing facility and confirmed. The accession numbers obtained were OP732981, OP732979 for *map*A and *ceu*E genes respectively.

A similar study was conducted on meat samples collected from Uttar Pradesh, India, by Singh *et al*. (2009) where out of the 300 samples collected, 73 per cent was *C. jejuni*. Similar findings were observed by Hagos *et al*. (2021) where out of the 210 samples collected from Ethiopia, 76 per cent was *C. jejuni*. In contrast to this result, Khan *et al*. (2018) concluded that in the *Campylobacter* spp. isolated from meat samples from North India, 74.55 per cent



P- Positive Control
N- Negative Control
S1, S2, S3, S4 - Samples

Fig.1. Agarose gel electrophoresis of PCR product – *16S r*RNA, *map*A and *ceu*E gene

were *Campylobacter coli,* and only 25.45 per cent were *C. jejuni*. Similarly, Du *et al*. (2018) concluded that in the *Campylobacter* spp. isolated from meat samples from Shanghai, 78.88 per cent were *Campylobacter coli* and only 21.19 per cent were *C. jejuni*.

The occurrence of these virulent genes in these organisms may have some correlation to the environment in which the animals are raised. Since we collected the beef samples from retail shops where they raised animals in pastures with poor hygienic conditions, the chance of colonisation of organisms in animals are more compared to dairy cattle maintained in good conditions.

Mostly, the source of Campylobacter in raw milk is faecal contamination from cattle. Therefore, the application of appropriate hygiene measures can largely prevent contamination. According to Beumer *et al*. (1988), the low rate of *Campylobacter* spp. in raw milk samples was attributed to the antimicrobial lactoperoxidase system in milk. Any change in the pH of the milk may inactivate the lactoperoxidase system and affects the milk quality. In this study, the samples were aseptically collected from hygienic milk societies and the active antimicrobial lactoperoxidase system in the samples attributes to the absence of organism. The difference in the hygienic measures, climatic conditions and variations in the availability of oxygen and temperature can also affect the survivability of this pathogen.

Conclusion

The occurrence of Campylobacter in food poses a potential public health risk. In this study none of the milk samples were positive for Campylobacter. The proper hygienic measures adopted during the production and handling of the milk prevents the contamination with organism. The main source of Campylobacter in meat is from slaughter house environment. Strict hygienic and sanitary operations should be followed throughout production and processing. Slaughterhouse personnel should be trained on hygienic carcass handling and standard food safety operations. The public should be made aware of the importance of clean milk and safe meat production to prevent the occurrence of campylobacteriosis through milk and meat.

Conflict of interest

The authors declare no conflict of interest.

References

Abri, R., Javadi, A., Asghari, R., Razavilar, V., Salehi, T.Z., Safaeeyan, F. and Rezaee, M.A. 2019. Surveillance for enterotoxigenic & enteropathogenic *Escherichia coli* isolates from animal source foods in Northwest Iran. *Ind J. Med. Res.***150**: 87p.

Athulya, T.R., Latha, C., Sunil, B., Deepa, J. and Shynu, M. 2021. Occurrence of *Campylobacter* spp. in duck and associated environmental samples in Thrissur district. *J. Vet. Anim. Sci*. **52**: 325-330.

Denis, M., Soumet, C., Rivoal, K., Ermel, G., Blivet, D., Salvat, G. and Colin, P. 1999. Development of am□PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli. Lett. Appl. Microbiol.* **29**:406-410.

Du, Y., Wang, C., Ye, Y., Liu, Y., Wang, A., Li, Y., Zhou, X., Pan, H., Zhang, J. and Xu, X. 2018. Molecular identification of multidrug-resistant *Campylobacter* spp. from diarrheal patients and poultry meat in Shanghai, China. *Frnt. Microbiol. **9***: 16-42.

Hagos, Y., Gugsa, G., Awol, N., Ahmed, M., Tsegaye, Y., Abebe, N. and Bsrat, A. 2021. Isolation, identification, and antimicrobial susceptibility pattern of *Campylobacter jejuni* and *Campylobacter coli* from cattle, goat, and chicken meats in Mekelle, Ethiopia. *PloS one*. **16**: 20p.

Hansson, I., Vagsholm, I., Svensson, L., Olsson, E. 2007. Correlations between *Campylobacter* spp. prevalence in the environment and broiler flocks. *J. Appl. Microbiol.* **103**: 640-649.

Igwaran, Aboi, and Anthony I. Okoh. 2020. Campylobacteriosis agents in meat carcasses collected from two district municipalities in the Eastern Cape Province, South Africa. *Foods* **2**: 203.

Joby, J.E. 2016. Occurrence of Campylobacter spp. in chicken egg production chain. M.V.Sc thesis, Kerala Veterinary and Animal Sciences University, Pookode, 64p.

Khan, J.A., Rathore, R.S., Abulreesh, H.H., Qais, F.A. and Ahmad, I. 2018. Prevalence and antibiotic resistance profiles of *Campylobacter jejuni* isolated from poultry meat and related samples at retail shops in Northern India. *Foodborne Pathog. Dis.* **15**: 218-225.

Korsak, D., Mackiw, E., Rozynek, E., Zyłowska, M. 2015. Prevalence of *Campylobacter* spp. in retail chicken, turkey, pork, and beef meat in Poland between 2009 and 2013. *J. Food Prot.* **78**: 1024-1028.

Linton, D., Owen, R.J. and Stanley, J. 1996. Rapid identification by PCR of the genus *Campylobacter* and of five *Campylobacter* species enteropathogenic for man and animals. *Res. Microbiol.* **147**: 707-718.

Modi, S., Brahmbhatt, M.N., Chatur, Y.A. and Nayak, J.B. 2015. Prevalence of *Campylobacter* species in milk and milk products, their virulence gene profile and anti-bio gram. *Vet. Wld*. **8**: 2p.

Narvaez-Bravo, C., Taboada, E.N., Mutschall, S.K. and Aslam, M. 2017. Epidemiology of antimicrobial resistant *Campylobacter* spp. isolated from retail meats in Canada. *Int. J. Food Microbiol.* **253**:43-47.

OIE. [Office International des Epizooties]. 2017. *Infection with C. coli and C. jejuni*. [Chapter 2.9.3.]. OIE Terrestrial Manual. World Organisation for Animal Health, 9p.

Singh, R., Singh, P.P., Rathore, R.S., Dhama, K. and Malik, S.V.S. 2009. Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in meat and carcasses collected from local poultry farms and retail shops of Bareilly, Uttar Pradesh, India. *Indian J. Comp. Microbiol. Immunol. Infect. Dis*. **30**: 35-58.

Swetha, S.V., Babu, A.J., Rao, T.M. and Kumar, E. 2015. Evaluation of various selective and non-selective broths for detection of *Listeria monocytogenes* in pork and for PCR compactibility. *Int. J. Adv. Res.* **3**: 316-327.

Vani, R.P., 2018. Identification of *Campylobacter* spp. critical control points in beef production chain. *M. V. Sc. thesis*, Kerala Veterinary and Animal Sciences University, Pookode, 96p.

Vivekanandhan, R., Sunil, B., Latha, C., Vrinda Menon, K., Ambily, R. and Gleeja, V.L. 2022. Occurrence of thermophilic *Campylobacter* spp. in pigs and the assessment of biosecurity measures employed at unorganized pig farms in Thrissur, Kerala. *J. Vet. Anim. Sci.* **53**: 602-608.

Wegmuller, B., Luthy, J. and Candrian, U. 2003. Direct polymerase chain reaction detection of *Campylobacter jejuni* and *Campylobacter coli* in raw milk and dairy products. *Appl. Environ. Microbiol.* **59**: 2161-2165.

WHO [World Health Organization]. 2020. WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2016-2020.

Zang, X., Kong, K., Tang, H., Tang, Y., Tang, H., Jiao, X. 2018. A GICA strip for *Campylobacter jejuni* real-time monitoring at meat production site. *Vet. Wld*. **98**:500- 505. ■