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Occurrence of thermophilic *Campylobacter* spp. in chicken meat breeds from an organised farm in Thrissur district#

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Abstract

Campylobacteriosis is one of the principal causes of gastroenteritis in the world. Poultry, being the natural reservoir, can harbour large number of Campylobacter in their caeca. The current study was carried out to assess the occurrence of thermophilic Campylobacter spp. in different meat type breeds in an organised farm. Out of the 120 cloacal swabs analysed, 31.67 per cent were found to be positive for Campylobacter spp. There was no significant difference in occurrence of Campylobacter spp. between different meat breeds. On molecular confirmation, majority of the isolates (71.05 per cent) were identified as Campylobacter jejuni and the rest as Campylobacter coli (28.95 per cent). The present study confirms a substantial degree of Campylobacter contamination in meat type chicken breeds in the farm. Good hygienic practices with better biosecurity measures and periodic screening of live poultry, poultry meat and its products are necessary to reduce the foodborne transmission of this pathogen to ensure safer food for consumers.

Keywords: *Campylobacter spp., meat type breeds, culture, PCR*

Globally, Campylobacter is the leading cause of bacterial gastroenteritis in humans. Foodborne outbreaks, which always pose a serious threat to mankind, have been associated with significant morbidity and mortality worldwide. Each year, 550 million people suffer from diarrhoeal diseases, with almost one in ten people falling ill due to campylobacteriosis (Nigusu *et al*., 2022).

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Campylobacter is a Gram-negative, slender, non-spore forming, bipolar flagellated, spiral or helical shaped bacteria. Thermophilic Campylobacter grow best at 42°C and include *C. jejuni*, *C. coli, C. lari* and *C. upsaliensis.* Campylobacteriosis could be associated with some major complications like Guillain-Barre Syndrome (GBS), Reactive Arthritis (REA), Inflammatory Bowel Disease (IBD), Irritable Bowel Syndrome (IBS) and Miller Fisher Syndrome (MFS) (Vivekanandhan *et al*., 2022). Consumption of contaminated poultry meat and meat products can account for most cases of human campylobacteriosis. *Campylobacter jejuni* is usually seen in 2–3 weeks old broiler flocks, which corresponds with a fall in maternal antibody titres (Deepa *et al*., 2022). The intestinal tract of chicken, especially the caecum and colon, harbours a large number of *Campylobacter* spp. *Campylobacter jejuni* belongs to the normal avian microbiota and despite a huge colonisation number of upto 10^8 Colony Forming Units (CFU) per gram of intestinal content, chicken generally remain asymptomatic (Konkel *et al*., 2007). Campylobacter infection has a complex epidemiology with diverse animal and environmental reservoirs along with numerous risk factors making them a serious public health threat. There is limited information available in Kerala regarding the occurrence of *Campylobacter* spp. in different meat type breeds in organised farm conditions. Hence, the present study was undertaken to assess the presence of Campylobacter in an organised farm in Thrissur district.

Materials and methods

Collection of samples

A total of 120 cloacal swab samples, 30 each from Gramasree, Red Cornish, Australorp and Rhode Island Red (RIR), were collected from an organised farm during a period of three months from December 2021 to February 2022. Swabs were transferred aseptically in Cary-Blair transport media under chilled conditions in thermo-cool containers to the laboratory of Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Mannuthy. Samples were processed within four hours of collection to ensure that the organisms remain viable and culturable.

Isolation and identification of Campylobacter spp.

Cloacal swabs were directly plated onto Blood-free Campylobacter Selectivity (modified Charcoal Cefoperazone Deoxycholate, mCCD) agar media supplemented with CAT selective supplement (FD 145), Campylobacter supplement V (FD 067) and Polymyxin B selective supplement (FD 003) as per the procedure described by Chon *et al*. (2012). It was then incubated under microaerophilic conditions in a CO_2 incubator with 10 per cent CO_2 at 42°C for 48 h. Isolates were characterised using biochemical tests like gram staining, oxidase test, catalase test, aerobic growth test, hippurate hydrolysis and indoxyl acetate test described by Tenover and Fennell (1992) and followed by molecular confirmation.

Multiplex PCR for identification of Campylobacter spp

The isolates obtained were further subjected to confirmation by multiplex PCR for genus and species identification of *Campylobacter* spp. as per Denis *et al.* (1999) with slight modifications. The isolated DNA, from colonies on mCCDA plates were screened for the presence of *Campylobacter* spp., targeting genus-specific *16S r*RNA gene. Species level identification was done by PCR for the detection of *C. jejuni* specific *map*A gene and *C. coli* specific *ceu*E gene. The primer sequences used in the study are depicted in Table 1.

The multiplex PCR was done with a reaction mixture in a final volume of 30µL, consisting of 3 µL of 10X PCR buffer (200M*m),* 2µL of $MgCl_2$ (25M*m),* 0.75 µL of *Taq* DNA polymerase (5 Units/µL), 2.50µL dNTP Mix (2 m*M*) and 2µL of *16S r*RNA (1 µL each forward and reverse primers), 2µL of *map*A (10pmoles/µL) and 2µL of *ceu*E (20pmoles/µL) and 5µL of eluted DNA (50ng/µL) and nuclease free water. The mPCR amplification cycle included initial denaturation at 95°C for 10 min followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 51.8°C for 1 min and extension at 72°C for 1 min. The final stage was an extension cycle at 72° C for 10 min. The PCR

Table 1. Primers used for identification of *Campylobacter*

Genes	Length (bases)	Primer sequence	Size (bp)	Ref.
*16S r*RNA F	18	5'-GGATGACACTTTTCGGAGC-3'	816	Linton *et al*. (1996)
*16S r*RNA R	18	5'-CATTGTAGCACGTGTGTC-3'		
*ceu*E F	23	5'-AATTGAAAATTGCTCCAACTATG-3'	462	Denis *et al*. (1999)
*ceu*E R	23	5'-TGATTTTATTATTTGTAGCAGCG-3'		
*map*A F	24	5'-CTATTTTATTTTTGAGTGCTTGTG-3'	589	Denis *et al*. (1999)
*map*A R	25	5'-GCTTTATTTGCCATTTGTTTTATTA-3'		

was carried out in an automated thermal cycler (Bio-Rad T100 Thermal Cycler, USA). The final PCR products were visualised in two per cent agarose gel using SYBR safe DNA gel stain solution.

Results and discussion

After direct plating onto p-MCCDA agar and following incubation, greyish, flat, spreading type, shiny, mucoid and moistened colonies with a tendency to spread, and with or without metallic sheen was observed (Fig.1). Out of 120 samples, 38 samples that were positive for Campylobacter on culture were further subjected to molecular confirmation.

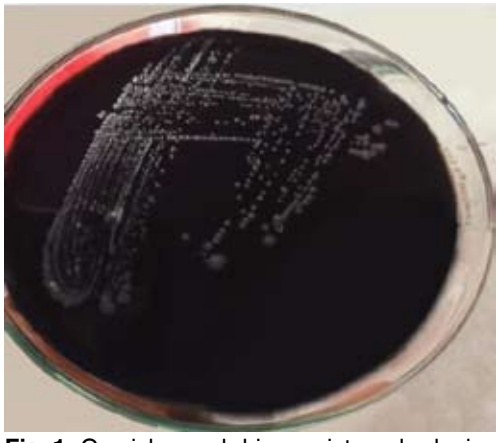

Fig. 1. Greyish round shiny, moistened colonies on p-MCCD agar

All the 38 isolates obtained were subjected to molecular confirmation by mPCR by targeting *16S r*RNA gene for genus identification and *map*A and *ceu*E for species level identification. An 816 bp amplicon was obtained upon PCR targeting *16S r*RNA gene, 589 bp amplicon was obtained for *map*A gene and 462 bp amplicons signaled *ceu*E gene (Fig. 2). The amplicons obtained consequent to PCR of species-specific *map*A gene, *ceu*E gene were purified and were outsourced for sequencing and obtained accession number of *C. jejuni*- OQ164769, *C. coli*- OQ164770.

The genus specific *16S r*RNA gene, for *Campylobacter* spp. was amplified in all the 38 isolates. The overall occurrence of *Campylobacter* spp. from cloacal swabs in the farm by culture and molecular methods was 31.67 per cent. Details of occurrence of *Campylobacter* spp. in cloacal swabs of different meat type breeds confirmed by molecular method is given in Table 2. There was no significant difference in the occurrence of *Campylobacter* spp. in different meat type breeds. The prevalence of Campylobacter obtained from meat type breeds in present study was in accordance with the results of Heuer *et al*. (2001) where they reported a 36.7

Table 2. Occurrence of *Campylobacter* spp. in different chicken meat breeds

Sl. No.	Samples analysed (n)	Meat type breeds	Positive samples		Chi-square value	p-value
			No.	Per cent		
1	30	Gramasree	9	30.00	.770	0.857^{ns}
2	30	Australorp	8	26.67		
3	30	Red cornish	11	36.67		
4	30	RIR	10	33.33		
Total	**120**		**38**	**31.67**		

Ns-nonsignificant at (p>0.05)

Table 3. Distribution of *C. jejuni and C. coli* in chicken meat breeds

Sl. No.	Breeds	Samples analysed	Positive for Campylobacter	*C. jejuni*		*C. coli*	
				Number	Per cent	Number	Per cent
1	Gramasree	30	9	7	77.78	2	22.22
2	Australorp	30	8	5	62.50	3	37.50
3	Red Cornish	30	11	7	63.64	4	36.36
4	RIR	30	10	8	80.00	2	20.00
	Total	**120**	**38**	**27**	71.05	**11**	28.95

per cent of occurrence of Campylobacter in cloacal swabs collected from broiler flocks in Denmark. A similar prevalence study was done by Alam *et al*. (2020) where they reported 48.4 per cent of conventional farms and 20.7 per cent of good practice broiler farms in Bangladesh was contaminated by Campylobacter. A slightly lower prevalence (28 per cent) from backyard chicken cloacal swabs from Thrissur, Kerala was reported by Jacob *et al*. (2017). On the contrary, a higher occurrence of Campylobacter, 50.9 in broiler cloacal swabs were observed in Malaysia by Singulingga *et al*. (2019). Baali *et al*. (2020) from Algeria recorded an incidence of 65 per cent, an incidence rate higher than that of the present study.

Among the positive 38 isolates, *C. jejuni* specific *map*A gene was amplified in 27 isolates (71.05 per cent) and *C. coli* specific *ceu*E gene was present in 11 isolates (29.4 per cent) (Table 3). This finding was in prefect tune with the result of Alam *et al*. (2020) where the authors identified 68 percent of isolates as *C. jejuni*. According to Schets *et al*. (2017) from Netherlands, all the Campylobacter isolates from caecal contents of broilers in a farm were *C. jejuni*. However, Pillay *et al*. (2020) reported a higher prevalence of *C. coli* (60 per cent) among the isolated samples from poultry farms of South Africa. Poultry is frequently colonised with *C. jejuni* (65–95 per cent), less often with *C. coli* and rarely with other *Campylobacter* spp. (Newell *et al*., 2001). The difference in occurrence of two Campylobacter species varies with region, species, climatic conditions and other environmental factors. The relatively lesser prevalence observed in different meat type breeds in the present study could be due to very good husbandry practices and proper biosecurity measures adopted in the organised farm and also could be due to seasonal variations. For the present study, samples were collected in the months of December to February, which could be correlated to the fact that lower incidence of Campylobacter was

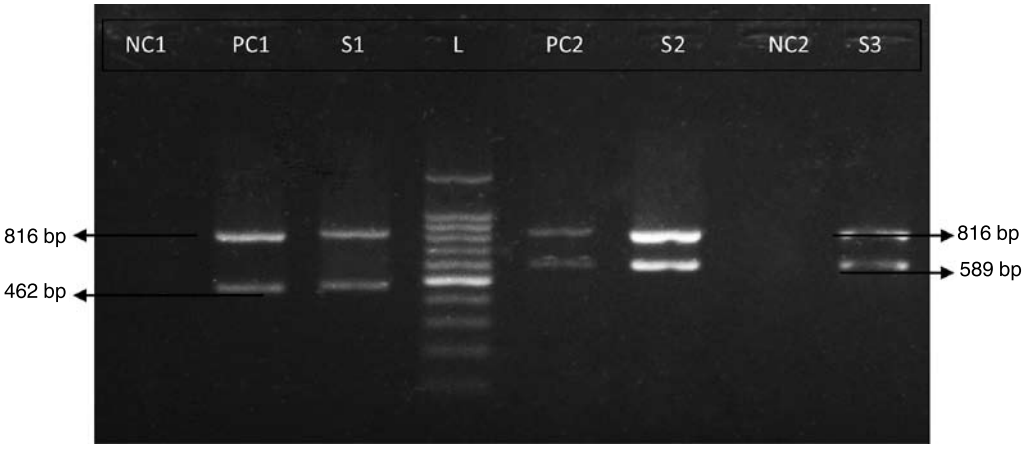

Fig. 2. Agarose gel electrophoresis of mPCR product- *16s r*RNA, *ceu*E, *map*A (PC1&PC2- Positive Control for *C. coli* & *C. jejuni,* L-100bp ladder, NC1&NC2-Negative Controls, S1,S2,S3 - Samples)

noted in winter months compared to summer (Baali *et al*., 2020). Frequent shedding of the organism in the faeces can contaminate feed, water and litter in the farm which can lead to rapid horizontal spread among the flock.

Thus, the present study confirmed the presence of Campylobacter contamination in cloacal swabs of meat type chicken which poses a great public health threat. Higher rate of contamination of caeca with this organism can lead to cross contamination during poultry processing and increase chances of risk to humans. Strict biosecurity measures, along with personal hygiene in poultry keepers need to be ensured through participatory training under a one health approach. Biosecurity and hygiene measures clearly have a great role in preventing flock colonisation in the field. Effective screening of poultry, poultry products and humans are much needed for the development of control strategies for this organism.

Conclusion

Campylobacter contamination was detected in cloacal swabs collected from different chicken meat breeds in an organised farm. This highlighted the importance of stringent hygienic practices and biosecurity measures along with effective monitoring which need to be followed, not only for prevention of foodborne infection but also for better production and performance of the poultry.

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Conflict of interest

The authors declare no conflict of interest.

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