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Occurrence of *Campylobacter* spp. in duck and associated environmental samples in Thrissur district#

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Abstract

Campylobacteriosis caused by Campylobacter spp. is considered as the most common cause of bacterial diarrhoea in humans across the globe. The current research was undertaken to assess the occurrence of Campylobacter spp. in duck and the associated environmental samples. Among 220 samples analysed, 7.73 per cent samples revealed the presence of Campylobacter spp. Majority of the samples contained C. coli (4.55 per cent) and C. jejuni was detected in 3.18 per cent of the samples. The present study revealed a high occurrence of Campylobacter spp. in duck rearing facilities in Thrissur district, Kerala. As the demand for duck products is increasing every year, the risk of contamination by Campylobacter spp. has to be viewed seriously. The study revealed the importance of multifaceted one health approach including human medicine, veterinary medicine, epidemiology, environmental hygiene, public health institutes and epidemiological surveillance agencies to control food-borne diseases and up-gradation of biosecurity measures.

Keywords: *Campylobacter spp. one health*

Running Title: Occurrence of *Campylobacter* spp. in duck of Thrissur district

Campylobacter spp. is considered as an important cause for foodborne gastroenteritis in humans worldwide which is mostly associated with *C. jejuni* and *C. coli* (Wesley *et al*., 2000). Sporadic outbreaks often occur by consumption of raw or undercooked poultry products. In humans, the disease caused by *Campylobacter* spp. is normally mild and self-limiting, but severe systemic

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infection can result in chronic sequelae such as Guillain-Barre syndrome (GBS), irritable bowel syndrome, Miller Fisher syndrome and reactive arthritis. The natural reservoirs of thermophilic campylobacters are free-living birds and commercial poultry. Despite duck being an important reservoir for *Campylobacter* spp. and a major part of modern Asian diet, information regarding the occurrence of the organism in duck is very limited. Therefore, the current investigation was designed with an objective to determine the occurrence of the *Campylobacter* spp. in duck rearing facilities.

Materials and methods

The current research was conducted to assess the occurrence of *C. coli* and *C. jejuni* in duck and environmental samples associated with duck rearing along with molecular confirmation of the positive isolates. The investigation was conducted for a period of 10 months from October 2019 to July 2020. A total of 220 samples from three different duck farms in and around Thrissur district were analysed during the study period. Samples consisted of duck cloacal swabs, soil and drinking water from duck farms. Details of samples are given in Table 1.

Isolation and identification of *Campylobacter* spp. from collected samples were carried out by selective enrichment followed by selective plating as recommended by Stern *et al*. (2001) and OIE Terrestrial Manual (2017) with necessary modifications. Cloacal swab was streaked onto modified Charcoal Cefoperazone Deoxycholate Agar plates supplemented with Polymyxin B (P-mCCDA). The plates were then incubated at 42°C for 48 h under microaerophilic conditions. Soil and drinking water samples were subjected to enrichment in mCCD (modified Charcoal Cefoperazone Deoxycholate) broth supplemented with CCDA selective supplement (FD 135). Incubation was done under microaerophilic conditions at 42°C for 48 h followed by selective plating onto mCCDA supplemented with CAT 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, flat, spreading type, shiny, mucoid and moistened colonies with tendency to spread and with or without metallic sheen were considered as *Campylobacter* spp. (Fig. 1).

The confirmation of *Campylobacter* spp. was carried out as per the procedure described by El-Adawy *et al.* (2012) with slight modifications by subjecting all the positive Campylobacter isolates to multiplex polymerase chain reaction. The positive isolates were further analysed for the presence of *16S rRNA* gene specific for *Campylobacter* genus and virulence gene *cad*F. *C. coli* and *C. jejuni* were identified by the detection of *ceu*E gene specific for *C. coli* and *map*A gene specific for *C. jejuni*. The target genes that were detected by mPCR and the primer sequences used in the study are shown in Table 2. A master mix was prepared for each *Campylobacter* spp. before setting up of the PCR reaction by combining the reagents as depicted in Table 3. Cycling conditions for mPCR are depicted in Table 4. The PCR products were stained with SYBR safe dye and detected by submarine gel electrophoresis.

Results and discussion

Cloacal swabs of duck

Per cent occurrence of *Campylobacter* spp. is given in Table 5. The presence of *Campylobacter* spp. in DF2 (22.5 per cent) was significantly higher ($p<0.05$) compared to DF3

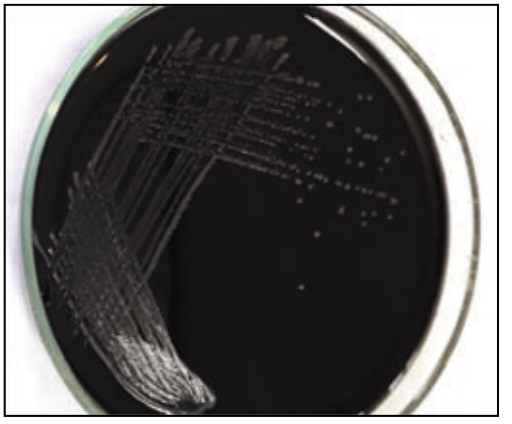

Fig. 1. Greyish, round, spreading type, shiny, moistened colonies of *Campylobacter* spp. on mCCD agar

Table 1. Details of sample collection

Sl. No.	Duck farm	Cloacal swabs	Soil	Water
1	DF1	40	15	15
2	DF2	40	15	15
3	DF3	40	20	20
Total		**120**	**50**	**50**

Table 2. Primers used for the identification of *Campylobacter* spp.

Primer	Primer sequence	Size (bp)	Reference
*16S r*RNA F	5'-GGATGACACTTTTCGGAGC-3'	816	Linton *et al*. (1996)
*16S r*RNA R	5'-CATTGTAGCACGTGTGTC-3'		
*cad*F F	5'-TTGAAGGTAATTTAGATATG-3'	400	Rozynek *et al.* (2005)
*cad*F R	5'-CTAATACCTAAAGTTGAAAC-3'		
*map*A F	5'-CTATTTTATTTTTGAGTGCTTGTG-3'	589	Denis *et al.* (1999)
*map*A R	5'-GCTTTATTTGCCATTTGTTTTATTA-3'		
*ceu*E F	5'-AATTGAAAATTGCTCCAACTATG-3'	462	Denis *et al.* (1999)
*ceu*E R	5'-TGATTTTATTATTTGTAGCAGCG-3'		

Table 3. Components of multiplex PCR mixture

Sl. No.	Name of the reagent	Stock Concentration	Quantity (µL)
1	Template DNA	50 ng/µL	5.00
2	10X PCR buffer	200 m*M*	3.00
3	$MgCl_2$	25 m*M*	2.00
4	*Taq* DNA polymerase	5 Units/µL	0.75
5	dNTP Mix	2 m*M* each	2.50
6	Forward primer of *16S r*RNA gene	10 pmoles/µL	1.00
7	Reverse primer of *16S r*RNA gene	10 pmoles/µL	1.00
8	Forward primer of *cad*F gene	20 pmoles/µL	1.00
9	Reverse primer of *cad*F gene	20 pmoles/µL	1.00
10	Forward primer of *map*A gene	10 pmoles/µL	1.00
11	Reverse primer of *map*A gene	10 pmoles/µL	1.00
12	Forward primer of *ceu*E gene	10 pmoles/µL	1.00
13	Reverse primer of *ceu*E gene	10 pmoles/µL	1.00
14	Nuclease free water		8.75
Total			**30.00**

(2.5 per cent). In the present study, 10 per cent of the total 120 cloacal swabs from ducks were positive for the organism which was in perfect tune with the results of Nor Faiza *et al*. (2013), where the authors reported an occurrence of the organism in 12 per cent cloacal swab samples of duck collected from Malaysia. However, Wei *et al*. (2014) in South Korea isolated *Campylobacter* spp. from 96.6 per cent of duck cloacal swabs. The farm with highest occurrence in the current study had muddy and wet floor compared to the other two farms with rather clean and dry environment.

Soil samples

In the present study, two (13.33 per cent) soil samples each from DF1 and DF2 carried the organism while none of the samples from DF3 harbored the same. There was no significant difference among the three duck farms regarding the occurrence of *Campylobacter* spp. in soil. The lower occurrence in the current research can be correlated with zero prevalence of the organism in soil samples reported by Adzitey *et al*. (2012) which could possibly be due to the poor

Table 4. Cycling conditions used for PCR of *16S rRNA*, *cad*F, *map*A and *ceu*E genes

Sl. No.	Steps	Temperature	Time (min)	No. of Cycles
1	Initial denaturation	95.0°C	10	1
2	Denaturation	94.0°C	1	30
3	Annealing	51.8°C	1	
4	Extension	72.0°C	1	
5	Final extension	72.0°C	10	1
6	Hold	4.0°C	10	

Table 5. Occurrence of *Campylobacter* spp. in duck rearing facilities

Sl. No.	Duck farm	Positive samples							
		Cloacal swab		Soil		Water		Total	
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
1	DF1	2	5.00[a, b]	2	13.33[a]	1	6.67[a]	5	7.14[a, b]
2	DF2	9	22.50[b]	2	13.33[a]	0	0.00[a]	11	15.71[b]
3	DF3	1	2.50[a]	0	0.00[a]	0	0.00[a]	1	1.25[a]
Total		**12**	**10.00**	**4**	**8.00**	**1**	**2.00**	**17**	**7.73**

Figures bearing same superscripts do not differ significantly ($p<0.05$)

Table 6. Distribution of genes in *Campylobacter* spp. isolates of duck rearing facilities

Sl. No.	Farm	No of isolates tested	Distribution of genes in the positive isolates (No.)			
			16S rRNA	***ceu*E**	***map*A**	***cad*F**
1	DF1	5	5	2	3	3
2	DF2	11	11	8	3	3
3	DF3	1	1	0	1	1
Total		**17**	**17**	**10**	**7**	**7**

survivability of the organism in soil. The results of the present study are contrary to that of Jensen *et al*. (2006) in Denmark, where the authors attributed the paddock contamination with *Campylobacter* spp. to the higher prevalence (35 per cent) in environmental samples. The occurrence recorded in the current study could be due to spill-over infections to soil from poultry reservoirs.

Water

Only one drinking water sample (collected from DF1) from duck rearing facilities was positive for the organism. There was no significant difference among the three duck farms regarding the occurrence of *Campylobacter* spp. in water. The results can be correlated with that of Adzitey *et al*. (2012), where none of the drinking water samples collected from duck farm harbored the organism. On the other hand, Van-Dyke *et al*. (2010) and Aung *et al*. (2015) recorded higher prevalence of *Campylobacter* spp. in water samples collected from Canada and Malaysia, respectively. The low recovery rate of the organism in the present research might be either due to the absence of the organism in water or the poor survivability of the organism in feed, soil, water and other surfaces exposed to sunlight, high oxygen tension and dry environment.

Overall occurrence in duck rearing facilities

Comparing the three duck farms under study, the occurrence of *Campylobacter* spp. in DF2 (15.71 per cent) was significantly higher ($p<0.05$) than DF3 (1.25 per cent). In farm DF1, 7.14 per cent samples were positive for the organism. The overall occurrence of *Campylobacter* spp. in duck rearing facilities was 7.73 per cent (Table 5). Kafshdouzan *et al*. (2019) reported an occurrence of the organism in 17.33 per cent of duck samples collected from Iran. An incidence of 73 per cent of *C. jejuni* was reported by Pacha *et al*. (1988) from migratory ducks in Pacific North American flyway. The low

recovery of the organism from majority of the samples in the current study could be possibly due to its poor survivability in the environment.

Molecular confirmation of the isolates from duck rearing facilities by mPCR

All the 17 isolates obtained from duck rearing facilities were subjected to mPCR for the simultaneous detection of *Campylobacter* genus specific *16S rRNA* gene, a conserved virulence *cad*F gene, *C. coli* specific *ceu*E gene and *C. jejuni* specific *map*A gene. The result of mPCR is depicted in Table 6.

Among the total 220 samples collected from duck rearing facilities, majority of the samples harbored *C. coli* (4.55 per cent) followed by *C. jejuni* (3.18 per cent). Nor-Faiza *et al*. (2013) also reported a higher occurrence of *C. coli* (88 per cent of total isolates) in duck samples collected from Malaysia compared to *C. jejuni*. On the other hand, according to Wei *et al*. (2014) and Jamali *et al*. (2015), *C. jejuni* was the predominant isolate from duck samples, accounting for more than 80 per cent of the positive isolates. The relative proportion of Campylobacter colonisation in gastrointestinal tract varies with different geographical area and type of host. This could be the reason for differences observed by several authors regarding occurrence of the two *Campylobacter* species.

Conclusion

All the three duck farms followed semi-intensive system of duck rearing, thus enhancing the chances of horizontal transmission of organism from the environment. Physical barriers that are capable of restricting the access to duck houses and external environment around the farms should be implemented to prevent the introduction of *Campylobacter* spp. via farmers, animals and visitors. Along with that, stringent biosecurity measures are required to prevent the contamination and propagation of organism among different flocks.

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Conflict of interest

The authors declare that they have no conflict of interest.

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