RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2022.53.4.

Occurrence of *Campylobacter* spp. in organised layer farms and associated environmental samples of Central Kerala#

C. P. Pravitha[1*], Jolly Deepa[2], C. Latha[3], B. Sunil[4] and R. Ambily[5]
Department of Veterinary Public Health
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651
Kerala Veterinary and Animal Sciences University, Kerala, India.

Citation: Pravitha,C.P., Jolly Deepa, Latha,C., Sunil,B. and Ambily,R. 2022. Occurrence of *Campylobacter* spp. in organised layer farms and associated environmental samples of Central Kerala. *J. Vet. Anim. Sci.* **53**(4):
DOI: https://doi.org/10.51966/jvas.2022.53.4.

Received: 27.06.2022 *Accepted: 30.08.2022* *Published: 31.12.2022*

Abstract

Campylobacteriosis caused by Campylobacter spp. is the prime cause of bacterial gastroenteritis worldwide. Chicken and other poultry birds act as the major reservoir for Campylobacter and thereby play a crucial role in the transmission of this zoonotic disease to humans. The current investigation was undertaken to study the occurrence of Campylobacter in organised layer farms and their associated environmental samples. A total of 260 samples comprising of cloacal swabs, feed, litter, soil, water and handwash of personnel were collected from two organised layer farms of Thrissur and Ernakulam districts. Isolation and identification of the organism by conventional culture technique followed by molecular confirmation of Campylobacter isolates using multiplex polymerase chain reaction (mPCR) revealed an overall occurrence of 17.31 per cent in layer farms. Majority of the isolates obtained in the study were C. jejuni (75.6 per cent) followed by C. coli (24.4 per cent). In the current scenario, where the consumer demand for chicken meat and eggs is continuously increasing, the occurrence of Campylobacter in layer farms needs to be addressed seriously. Appropriate interventions and control measures at farm level and also across the poultry production chain is necessary to minimise the impact of the disease on human health and economy.

Keywords: *Campylobacter spp., layer birds, conventional, PCR*

Campylobacteriosis, the prime cause of bacterial gastroenteritis worldwide, is an important foodborne illness resulting in 400 to 500 million cases of infection every year across the world (WHO,

#Part of M.V.Sc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

1. M.V.Sc scholar

2. Assistant Professor

3. Director (Academics and research),KVASU

4. Professor and Head

5. Assistant Professor, Department of Veterinary Microbiology

**Corresponding author: pravithaprakash94@gmail.com, Ph.8075825472*

2020). The disease is caused by *Campylobacter* spp. predominantly, *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*). It is an important public health problem with extensive animal and environmental reservoirs. The disease is self-limiting and mild in humans, but can be burdened with chronic sequelae like Guillain Barre syndrome, Miller Fisher syndrome, Irritable Bowel Syndrome and reactive arthritis. Poultry species act as the major reservoir for thermophilic *Campylobacter* spp. like *C. jejuni*. Despite its extensive colonisation in chicken caeca and intestinal contents, Campylobacter produces little or no clinical signs in poultry. Humans are exposed to the infection while handling and by consumption of uncooked poultry meat and meat products. Meat and eggs from poultry constitute an important part of the modern Asian diet. However, information regarding the occurrence of Campylobacter among layers, breeder flocks and broiler birds is often limited. Considering these factors, the present study was designed with an objective to study the occurrence of Campylobacter in the layer farms and associated environmental samples of Thrissur and Ernakulam districts of Kerala.

Materials and methods

The current research was conducted for a period of 10 months from June 2021 to March 2022. A total of 260 samples, 130 each were collected from two organised layer farms, one each from Thrissur and Ernakulam districts. The samples collected included 120 cloacal swabs from layer birds above 72 weeks of age, 30 samples each of feed, litter, drinking water, handwash of farm personnel and 20 soil samples. Details of samples collected are given in Table 1. Isolation and identification of *Campylobacter* spp. from samples were carried out according to OIE Terrestrial Manual (2017) with necessary modifications. The cloacal swab samples were directly swabbed onto modified Charcoal Cefoperazone Deoxycholate Agar plates supplemented with Polymyxin B (P-mCCDA). All other environmental samples were subjected to enrichment in mCCD (modified Charcoal Cefoperazone Deoxycholate) broth supplemented with CCDA selective supplement (FD 135) at 42°C for 48 h under microaerophilic conditions. This was followed by selective plating onto mCCDA supplemented with Campylobacter supplement

Table 1. Details of samples collected from layer farms

Sl. No.	Layer farm	Cloacal swabs	Type of Samples				
			Feed	Litter	Drinking water	Handwash	Soil
1	F1	60	15	15	15	15	10
2	F2	60	15	15	15	15	10
	Total	**120**	**30**	**30**	**30**	**30**	**20**

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

Gene	Primer sequence	Size (bp)	Reference
*16S r*RNA	F – 5'-GGATGACACTTTTCGGAGC-3'	816	(Linton *et al*., 1996)
	R - 5'-CATTGTAGCACGT GTGTC-3'		
*lpx*A *C. jejuni*	F-5'-ACAACTTGGTGACGA TGTTGTA-3'	331	(Klena *et al*., 2004)
	R-5'CAATCATGDGCDAT ATGASAATAHGCCAT -3'		
*lpx*A *C. coli*	F-5'-AGACAAATAAGAG AGAATCAG -3'	391	(Klena *et al*., 2004)
	R-5'-CAATCATGDGCDAT ATGASAATAHGCCAT-3'		

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

V (FD 067), Campylobacter growth supplement (FD 009), CAT selective supplement (FD 145), and Polymyxin B selective supplement (FD 003). The plates were incubated under microaerophilic conditions consisting of five per cent carbondioxide at 42°C for 48h. Greyish, flat, round, spreading type, shiny, mucoid and moistened colonies with tendency to spread and with or without metallic sheen were considered to be presumptive of *Campylobacter* spp. (Fig. 1). The biochemical characterisation of the suspected colonies was performed by the tests described by Tenover and Fennell (1992).

The molecular confirmation of isolates was carried out by polymerase chain reaction (PCR) targeting the *16S r*RNA gene specific for Campylobacter genus. For species level identification, a multiplex PCR (mPCR) assay was optimised for the two thermophilic species of Campylobacter *viz*., *C. jejuni* and *C. coli*. Multiplex PCR was performed in a final volume of 25 µL reaction mixture using 4 µL of extracted DNA as template. The reaction mixture included, 3 µL of 10X PCR buffer, 2 µL of 25mM MgCl2, 0.50 µL of *Taq* DNA polymerase (5 Units/µL), 0.50 µL of dNTP Mix (10mM), 1 µL (15 pmoles/ µL) each of forward and reverse primers of *lpx*A gene and nuclease free water made upto 25 µL total volume. The lipid A gene, *lpx*A was targeted in this assay, which produced discriminatory band of 331 bp for *C. jejuni* and 391 bp for *C. coli*. The cycling conditions for *lpx*A gene was optimised at an annealing temperature of 50°C. Details of primers used are given in Table 2.

Results and discussion

The cloacal swab samples procured from layer birds of F1 yielded more Campylobacter isolates (38.33 per cent) than F2 (30 per cent) as shown in Figure 2. The overall occurrence of Campylobacter in the cloacal swabs of layers from both districts was 34.17 per cent. This finding was in accordance with Al-Natour *et al*. (2018) from Northern Jordan, where the author reported a flock level prevalence of 40 per cent in the cloacal swabs of layer chickens. On the contrary, Kalupahana *et al*. (2013) and Schets *et al*. (2017) from Sri Lanka and Netherlands found a higher occurrence of *Campylobacter* spp. *i.e.,* 64 and 97 per cent, respectively, in the cloacal swab samples of layers. The lower prevalence obtained from layer chicken in the present study could be due to development of resistance to Campylobacter colonization towards the end of laying period. Campylobacter could be isolated from three drinking water samples and one soil sample collected from farms F1 and F2. Overall occurrence in drinking water samples was 10 per cent. Similar results were reported previously by Athulya (2021), where the organism was found in seven per cent of water samples collected from the duck rearing facilities of Thrissur district, Kerala. In the present study, water samples from the drinking water trough placed for layer birds within the poultry house were found to be positive for the organism. Campylobacter surviving in the crop contents of the birds (Jeffrey *et al*., 2001) might have caused contamination of water in the trough and subsequent cross-contamination of the entire flock. The organism was not detected in any of the other poultry rearing related environmental samples such as feed, litter or handwash of farm personnel. These findings were similar to the reports by Vivekanandhan (2018), where none of the feed, litter and handwash samples collected from the bird rearing facilities of Bareilly, Uttar Pradesh revealed the presence of Campylobacter. Nonetheless, findings of the present study were contrary to the results of Alam *et al*. (2020) from Bangladesh, where they found a higher occurrence of Campylobacter *i.e*., 18.8 per cent in 64 feed samples analysed. The zero prevalence of Campylobacter reported from feed samples of F1 and F2 could be due to the low moisture content of poultry feed

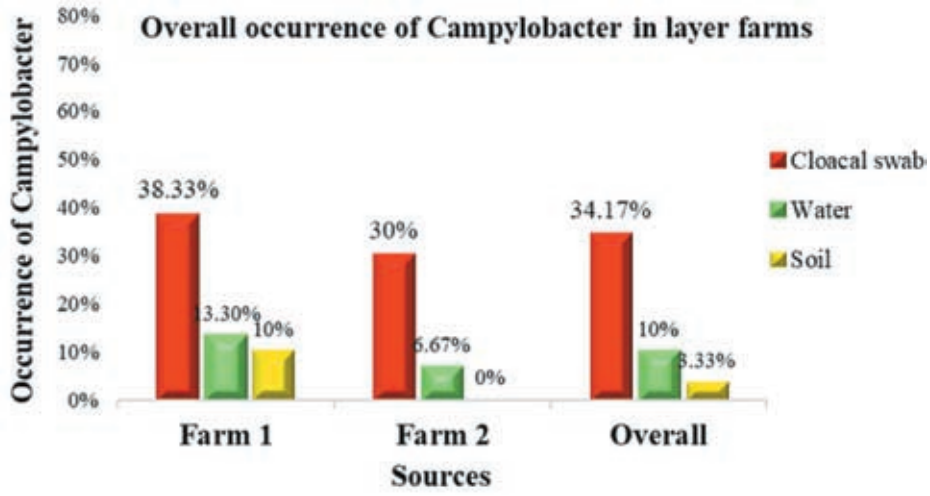


Fig. 2. Overall occurrence of Campylobacter in layer farms

and inability of Campylobacter to withstand dehydration. Campylobacter adopts a viable but non-culturable (VBNC) state under stressful environmental conditions, which cannot be revived by enrichment, thereby limiting its detection by routine culture methods.

Overall occurrence of *Campylobacter* spp. in layer farms in the present study was 17.31 per cent. There was statistically significant difference in the occurrence of organism in the cloacal swabs (34.17 per cent) and environmental samples (2.8 per cent). These can be correlated with the findings of Joby (2016) where the cloaca of birds was identified as one of the most important contamination point for Campylobacter in poultry production chain. The occurrence of Campylobacter in the cloaca of birds can result in contamination of egg surface at the point of lay. These findings were in concordance with the results of Savita (2018) where she reported an overall prevalence of Campylobacter in 6.7 per cent in retail chicken eggs examined from central Kerala.

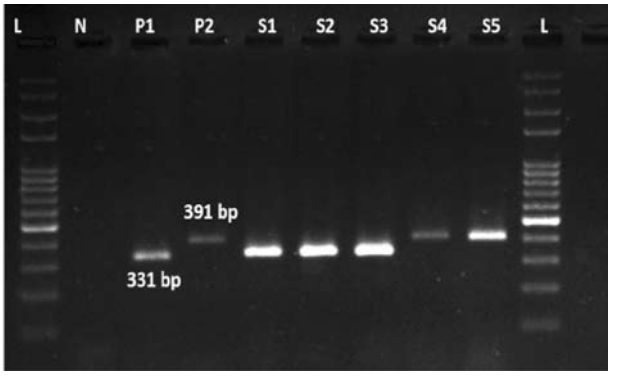
Molecular confirmation of Campylobacter revealed all 45 isolates obtained in the present study to be carrying genus specific *16S r*RNA gene. Species identification by mPCR revealed that majority of the isolates were *C. jejuni* (75.6 per cent) (Fig. 3) followed by *C. coli* (24.4 per cent). A similar distribution of *Campylobacter* spp. with high prevalence of *C. jejuni* was reported by Kabir *et al*. (2014) where 75 per cent isolates in their study were *C. jejuni.* The distribution of *Campylobacter* spp. in samples from the farms obtained is given in Table 3.

Conclusion

The current investigation revealed layer chicken as a possible source for *Campylobacter* spp. like *C. jejuni* and *C. coli.* This finding signifies the relevance of personnel hygiene measures and biosecurity interventions at farm level to limit the possible spread of infection to humans through the poultry production chain. The persistence of Campylobacter in layer hens towards the end of their laying cycle as suggested in the current study needs to be investigated further as this could lead to contamination of the slaughterhouse environment and chicken

Table 3. Distribution of *Campylobacter* spp. in layer farms

Sl. No.	Farm	No. of isolates	Distribution of genes in Campylobacter isolates		
			***16Sr* RNA**	***lpx*A (*C. jejuni*)**	***lpx*A (*C. coli*)**
1	F1	26	26	20	6
2	F2	19	19	14	5
	Total	**45**	**45**	**34**	**11**



L-100 bp ladder

N- Negative Control

P1- Positive Control (*C. jejuni*)

P2 – Positive control (*C. coli*)

S1, S2, S3, S4, S5 – Samples

Fig. 3. Detection of *lpx*A gene of *C. jejuni* and *C. coli* by mPCR

carcasses after the culling of such birds as spent hens.

Acknowledgement

The present work was carried out under the ICAR funded project 'Outreach Programme on Zoonotic Diseases'. The authors are thankful to the ICAR for funding this research and for providing the facilities required.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Alam, B., Uddin, M., Mridha, D., Akhter, A.H.M., Islam, S.K., Haque, A.K.M. and Kabir, S.M. 2020. Occurrence of Campylobacter spp. in selected small scale commercial broiler farms of Bangladesh related to good farm practices. *Microorganisms*. **8**: 1778.

Al-Natour, M.Q., Al-Aboudi, A.R., Alshehabat, M.A. and Tamimi, M.K. 2018. Prevalence and risk factors associated with *Campylobacter* among layer farms. *Vet. Sci. Dev*. **8**: 6430.

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.

Jeffrey, J.S., Tonooka, K.H. and Lozanot, J. 2001. Prevalence of *Campylobacter spp*. from skin, crop, and intestine of commercial broiler chicken carcasses at processing. *Poult. Sci.* **80**: 1390-1392.

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.

Kabir, S.M.L., Islam, J., Suman, M.H., Khan, M.S.R. and Yamasaki, S. 2014. Isolation, identification and antimicrobial susceptibility profiles of *Campylobacter* spp. with assessment of their risk factors in broiler flocks of Bangladesh Agricultural University Poultry Farm. *J. Basic Appl. Sci. Res*. **4**: 160-168.

Kalupahana, R.S., Kottawatta, K.S.A., Kanankege, K.S.T., Van Bergen, M.A.P., Abeynayake, P. and Wagenaar, J.A. 2013. Colonization of *Campylobacter* spp. in broiler chickens and laying hens reared in tropical climates with low-biosecurity housing. *Appl. Environ. Microbiol*. **79**: 393-395.

Klena, J.D., Parker, C.T., Knibb, K., Ibbitt, J.C., Devane, P.M., Horn, S.T., Miller, W.G. and Konkel, M.E. 2004. Differentiation of *Campylobacter coli, Campylobacter jejuni, Campylobacter lari* and *Campylobacter upsaliensis* by a multiplex PCR developed from the nucleotide sequence of the lipid A gene

*lpx*A. *J. Clin. Microbiol.* **42**: 5549-5557.

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.

OIE. 2017. *Terrestrial Manual*. (Chapter 2.9.3.). *Campylobacter coli* and *C. jejuni*. World Organization for Animal Health, 9p.

Savita, R.K., Latha, C., Sunil, B. and Sunanda, C. 2018. Occurrence of *Campylobacter* spp. in chicken egg from central Kerala. *J. Vet. Anim. Sci.* **49**: 53-58.

Schets, F.M., Jacobs-Reitsma, W.F., van der Plaats, R.Q., Heer, L.K.D., van Hoek, A.H., Hamidjaja, R.A., de Roda Husman, A.M. and Blaak, H. 2017. Prevalence and types of Campylobacter on poultry farms and in their direct environment. *J. Wat. Hlth*. **15**: 849-862.

Tenover, F.C. and Fennell, C.L. 1992. The genera *Campylobacter* and *Helicobacter*. In: Balows, A., Truper, H.G., Dworkin, M., Harder, W. and Schleifer, K. (eds.), *the prokaryotes*. (2nd Ed.). V. 4, New York, Spinger- Verlag. pp. 3488-3508.

Vivekanandhan, R. 2018. Antimicrobial resistance profile of thermophilic *Campylobacter* spp. from different poultry settings. *M.V.Sc thesis*, Indian Veterinary Research Institute, Izatnagar, 100p.

WHO [World Health Organisation]. 2020. WHO-*Campylobacters* Fact Sheet. Available https://www.who.int/news-room/fact-heets/detail/campylobacter [26 August 2022]. ■