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PREVALENCE OF *CAMPYLOBACTER* SPP. IN WATER BODIES OF CENTRAL KERALA

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ABSTRACT

Campylobacteriosis is one among the leading causes of bacterial gastroenteritis worldwide. The present study was undertaken to evaluate the occurrence of *Campylobacter* spp. in water bodies in central Kerala by conventional plating technique using Blood-free campylobacter broth and modified Charcoal Cefoperazone Deoxycholate agar in combination with multiplex polymerase chain reaction (mPCR). The influence of physicochemical parameters of water like pH, conductivity, temperature, total dissolved solids (TDS), salinity, resistivity, dissolved oxygen and hardness on this organism was also evaluated. *Campylobacter* spp. was detected in 54.76, 16.67, 6.67, 6.67, 30.0 and 10.0 per cents of streams/rivers (42), ponds (30), lakes (30), wells (30), brackish waters (30) and seawater (coastal-15 and deep-15), respectively, by direct mPCR of broth enriched samples. The predominant species was *Campylobacter jejuni*, followed by *Campylobacter coli* in rivers/streams. This study revealed a higher degree of turbidity in river/stream, hardness in pond water as well as resistivity, electrical conductivity and TDS in brackish waters and higher electrical conductivity in seawater, which are usually less conducive for survival of the organism. An alkaline pH in lake water favoured the survival of the organism. *Campylobacter* spp. in water bodies indicate that these can act as possible sources for transmission of food-borne campylobacteriosis.

Keywords: Campylobacter, Waterbodies, Physicochemical, Conventional, PCR

INTRODUCTION

Campylobacter spp. are known to be ubiquitous with the identified reservoirs being birds, domestic and wild animals, surface and ground fresh water, salt water, milk, soil and sewage. Campylobacteriosis is on the rise, not only on account of unreported cases but also population strength

and type, varied public health standards, food safety practices, surveillance systems; limited sensitivity in pathogen detection methods, intervention strategies as well as it's geographically varied prevalence in natural reservoirs (Hakeem and Lu, 2021). Though Campylobacter has a wide ranging animal reservoir with poultry and pigs being the two primary ones, humans are usually infected by this zoonotic pathogen by way of contaminated food and water (Igwaran and Okoh, 2019).

Various environmental water sources like rivers, streams, lakes, wells and coastal waters, can serve as contamination points either as a result of direct faecal droppings from birds and mammals (agricultural or wild) or with agricultural land runoff and improperly treated or untreated waste-water (Pitkanen, 2013). Despite being unable to multiply outside a host, the organism can survive in a number of environmental sources (Pitkanen and Hanninen, 2017). Survivability is dependent on species and environmental conditions like oxygen, light, temperature, biotic interactions and nutrient concentrations, the precise role of which, in the complex and diverse epidemiology of campylobacter infection, is still not fully known (Whiley *et al.*, 2013). Therefore, the present study was taken up to bring forth the public health significance of Campylobacter in water bodies in central Kerala.

MATERIALS AND METHODS

The present study was carried out to determine the extent of occurrence of *Campylobacter* spp. in the water bodies of central Kerala. All the samples were analysed for the presence of the organism and the isolates were identified. Samples were subjected to molecular detection of *Campylobacter* spp. directly from the initial enriched broth and the isolates obtained by culture methods were also subjected for confirmation by multiplex polymerase chain reaction for species identification as well as to detect the presence of the virulence genes. The total viable count and coliform count were also evaluated and the correlation with the presence of the organism was also assessed. The influence of physicochemical parameters of water like pH, conductivity, temperature, total dissolved solids (TDS), salinity, resistivity, dissolved oxygen (DO) and hardness on this organism was also evaluated.

Collection of Water from Sea and Freshwater Bodies*:* Water samples (n = 162) were collected from fresh-water bodies (42 from streams and rivers and 30 each from ponds, lakes and wells) and brackish waters (n = 30) from Central Kerala, *i.e.,* Thrissur and Ernakulam districts. A minimum of 30 sea-water samples (15 each from coastal and deep sea-waters) from the aforementioned two districts were

also collected. Approximately 500 mL of each water sample were collected in sterile sample bottles. River-water samples were collected from the rivers Bharathapuzha, Chalakudy, Choondal, Karuvannur, Kurumali, Manali and Puzhakkal, in and around central Kerala.

***Processing of samples**:* Isolation and identification of *Campylobacter* spp. from the samples were carried out by selective enrichment followed by selective plating as recommended by Stern *et al*. (2001) and OIE (2017) with necessary modifications. The selective enrichment of the samples was carried out in Blood Free Campylobacter (mCCD) broth with CCDA selective supplement (FD 135) under microaerophilic conditions in a CO_2 incubator (10 per cent CO_2 and 5 per cent oxygen) at 42 ^{0}C for 48 h. The organism was isolated from sea and river-water by subjecting 100 mL to membrane filtration through cellulose ester filters (MF-Millipore membrane filter) of 0.22 µm pore size and 47 mm diameter. The filter paper was then completely immersed in 90 mL of mCCD broth for isolation of *Campylobacter* spp. All the broth enriched samples were subjected to multiplex PCR to detect *Campylobacter* spp.

Loopful of the samples in mCCD broth were selectively plated onto Blood Free Campylobacter Selectivity (modified Charcoal Cefoperazone Deoxycholate) agar (mCCDA) 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) and then incubated under microaerophilic conditions. Greyish, flat, spreading type, shiny, mucoid and moistened colonies with tendency to spread, and with or without metallic sheen were selected for further characterisation.

For phenotypic confirmation, five or more suspected colonies from mCCD agar plates were subjected to further characterisation and identification by cultural, morphological and biochemical reactions. Molecular characterisation was performed by mPCR, targeting the presence of genus-specific *16S rRNA*, *C. jejuni* specific *map*A, *C. coli* specific *ceu*E genes and the virulence gene, *cad*F (Table 1).

Cyclic conditions used for multiplex PCR include Initial denaturation at 95 ^{0}C for 10 min, 30 cycles of Denaturation at 94 ^{0}C for 1min, Annealing at 51.8 ^{0}C for1min and Extension at 72 ^{0}C for 1min, Final extension at 72 ^{0}C for 10min and Holding at 4 ^{o}C for 10min. Subsequent to electrophoresis run, the gel was visualised and the images were documented on gel documentation system (Syngene, USA).

Table 1. Primers used for the PCR identification of *Campylobacter* spp.

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

The physico-chemical characteristics *viz.,* pH, salinity, electrical conductivity, dissolved oxygen and BOD of water was detected using multiparameter water analyser (Thermo USA, Thermo Fischer Scientific, Singapore) in the Laboratory of the Department of Veterinary Public Health. Data were subjected to statistical analysis.

RESULTS AND DISCUSSION

Over the last few decades, rapid urbanisation, increasing population and industrialisation have resulted in an increase in use of ground water resources in the State. Normally, Kerala receives an annual rainfall of 3060 mm, during the southwest monsoon period (May to September), followed by the northeast monsoon in November and December (KSCSTE, 2021).

Thisstudyprovidesdata/information on the occurrence of campylobacter in surface waters, which are used for domestic purpose, irrigation or as drinking water source. Campylobacter is quite often associated with instances of water-borne disease, with and without clinical symptoms and hence it is important to have up-to-date information on the prevalence of this pathogen to study it's epidemiology in various regions. The last two decades has witnessed thermophilic *Campylobacter* spp. as one of the prime causes of bacterial gastroenteritis in humans. Important sources for contamination of water can be faeces of birds, domestic and wild animals, agricultural runoff and municipal sewage discharges (Jones, 2001). Campylobacter readily tend to form viable but not culturable (VBNC) cells, outside the gastrointestinal tract and on exposure to environmental conditions (Rollins and Colwell, 1986). A comparative assessment was performed to study the occurrence of *Campylobacter* spp. as determined using culture (Fig. 1 and 2) and PCR based methods (Fig. 3).

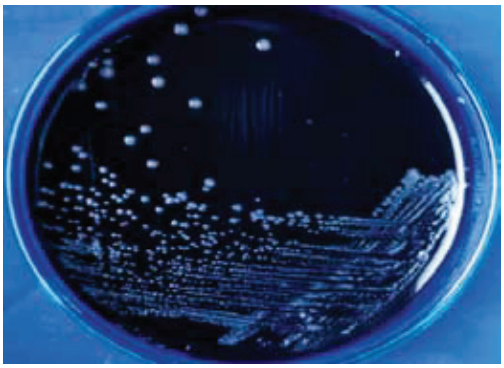


Fig. 1. *Campylobacter* spp. colonies on P- mCCD agar

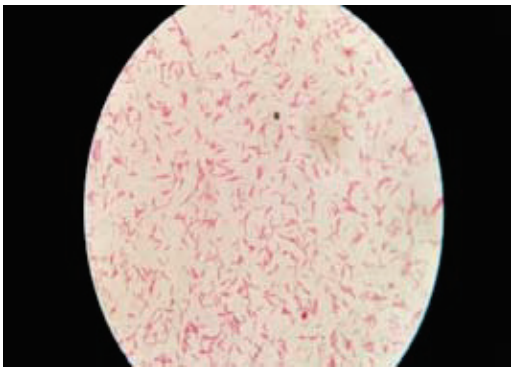
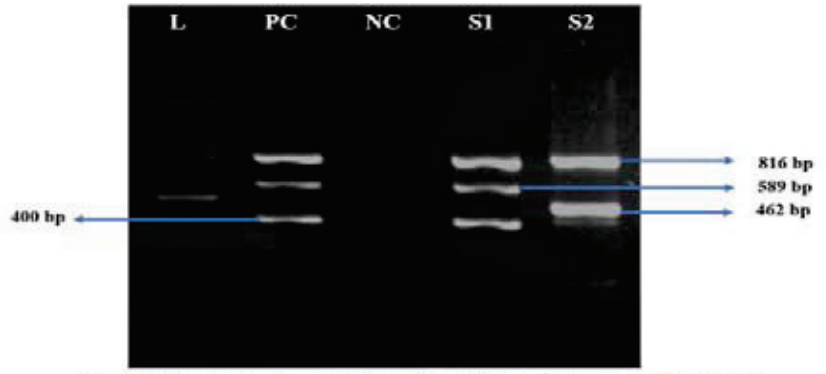


Fig. 2. Gram stained *Campylobacter* spp. (100X magnification)



PC - Positive control (*C. jejuni* NCTC 11168); NC - Negative control; S1 & S2 – Positive samples: L – Molecular ladder (100bp)

Fig. 3. Multiplex PCR profile *Campylobacter* spp. using *16S* rRNA, *mapA*, *ceuE* and *cadF* genes

The presence of campylobacter organism was detected in 54.76, 16.67, 6.67, 6.67, 30.0 and 10.0 per cents of streams/ rivers, ponds, lakes, wells, brackish and sea waters, respectively, by direct mPCR of the broth enriched samples. The overall occurrence in fresh surface water and seawater is as presented in table 2.

The prevalence of 25-55 per cent in ponds/lakes/streams in central Washington reported by Carter *et al.* (1987), 70 per cent in lakes/rivers in Poland by Popowski *et al.* (1997), 60 per cent in rivers, 75 per cent in ground water and 29.2 per cent in drinking water by Savill *et al.* (2001) in New Zealand are much higher than was obtained in this study from similar sources.

A lower prevalence than observed in the present study was reported by Daczkowska-Kozon and Brzostek-Nowakowska (2001) in surface water bodies (19.7 per cent) and river (19.7 per cent) in Western Pomerania, Moore *et al.* (2001) in domestic drinking water (2.2 per cent), environmental lake (4.3 per cent) in Ireland, Yaman *et al.* (2005) in lake (4.76 per cent), streams (14.28 per cent), drinking water (0 per cent) in Turkey,

Table 2. Distribution of *Campylobacter* spp. in water bodies in Thrissur and Ernakulam districts

Surface Water-bodies	No. of Direct PCR/Colony PCR samples in surface water-bodies								TOTAL		Overall total positive
	Thrissur				Ernakulam						
	CJ	CC	Mixed	Others	CJ	CC	Mixed	Others	Direct PCR	Colony PCR	
Streams and Rivers (42)	4/2	1/2	4/0	1/1	3/6	4/6	1/0	1/1	19	18	23
Ponds (30)	0/0	1/0	0/0	1/1	1/1	0/0	0/0	2/1	5	3	5
Lakes (30)	1/0	0/0	0/0	0/0	1/0	0/0	0/0	0/0	2	0	2
Wells (30)	1/1	1/1	0/0	0/0	0/0	0/0	0/0	0/0	2	2	2
Brackish water (30)	1/0	1/0	0/0	2/0	0/0	0/0	0/0	5/0	9	0	9
Deep(15)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0	0	0
Coastal (15)	1/0	0/1	0/0	1/0	0/0	0/0	0/0	0/0	2	1	3
TOTAL (162)	8/3	4/4	4/0	5/2	5/7	4/6	1/0	8/2	39	24	44

CJ- *Campylobacter jejuni*; CC- *Campylobacter coli*

Table 3. Physico-chemical parameters of water from water bodies

	Physicochemical parameters (Range)								
Surface Water-bodies	pH	Temperature (^{0}C)	Conductivity (µS/m)	TDS (ppm)	Salinity (ppt)	Resistivity (Ωm)	DO (ppm)	Hardness (N/mm^2)	Turbidity (NTU)
Streams and Rivers (42)	6.1-8.7	27.6-29.8	29.9-413.6	15.59-197.5	0.013-0.283	1.09-27.92	4.8-20.65	25-100	2.19-7.89
Ponds (30)	5.43-8.49	27.4-29.7	73.56-446.3	36.03-218.6	0.04-0.216	2.24-13.57	2.55-91.3	25-75	0.85-7.54
Lakes (30)	6.1-7.24	27.7-29.9	44.6-130.5	21.88-64.03	0.029-0.066	7.66-22.37	3.84-10.33	*	*
Wells (30)	6.15-7.53	26.5-30.1	66.8-614.4	32.61-300.4	0.03-0.24	1.27-14.46	2.06-31.9	*	*
Brackish water (30)	6.3-8.2	27.1-29.2	3.5-1808	1.7-881.7	0.91-19.2	34.2-553.1	5.4-10.35	100-1000	*
Seawater Deep(15) Coastal (15)	7.2-8.1	27.8-29.3	19.91-50.82	9.75-25.08	11.89-33.79	19.5-50.23	5.7-10.03	*	*

*- Not evaluated

Ghane *et al.* (2012) in coastal seawater (2.66 per cent) in Caspian sea, Khan and Edge (2013) in beaches (12 per cent and river (14 per cent) in Canada, Srigowthami (2013) in water (20 per cent) in TamilNadu, Sharma *et al.* (2016) in surface water (0 per cent) in Agra, Vani *et al.* (2018) in potable water (4 per cent) in Thrissur and Ferrari *et al.* (2019) in ponds (33.33 per cent) in Sweden. These organisms tend to revert to a VBNC form in water, which account for the lesser number of isolates obtained in this study. Horman *et al.* (2004) reported 17.3 per cent in lakes and rivers in Finland and Szczepanska *et al.* (2017) as 16.8 per cent in surface water in Poland, which are

consistent with the present results. All this emphasises the importance and significant role of surface water as a potential source of *Campylobacter*.

Polymerase chain reaction analysis aided in the detection of campylobacter at low concentrations from multiple sources. To enable better detection by culture-based methods, samples were enriched in CCD broth. With an enrichment-PCR method, high levels of thermophilic campylobacter, with detection rates of 60 and 75 per cents were observed in river and shallow ground water (Savill *et al.*, 2001) in New Zealand. The detection rate of *C. jejuni* in this study was higher in running water, *i.e.,* river, which is in agreement with Kemp *et al.* (2005), where the authors observed that *C. jejuni* was commonly isolated from trough and running water sources, while *C. coli* was isolated from standing water. Yaman *et al.* (2005) reported 4.76 per cent of *C. jejuni* in lakes and 14.28 per cent *C. jejuni* occurrence in streams in Turkey. The higher detection (6.67-54.76 per cent) in the present study is probably a reflection of the quality of the surface water in these tropical regions, particularly in India, where agriculture and animal husbandry go hand-in-hand. The practice of dumping wastes and effluents into water bodies can also contribute to the presence of the organism in the water bodies. These results highlight the fact that proper treatment measures have to be promulgated to encourage the use of treated quality-tested water for the general population. The higher occurrence observed in rivers was probably the result of VBNC cells, which may remain in water for weeks to months (Rollins and Colwell, 1986) and also the dead cells or free DNA in the water samples.

The virulence gene, *cad*F, of *Campylobacter* spp. could be detected in 10 of the 14 *C. jejuni* isolates, 3 of the 10 *C. coli* isolates, 3 of the 5 mixed isolates and 4 of the 12 other *Campylobacter* spp. isolates, while it could be detected in both of the *C. jejuni* and *C. coli* isolates from seawater.

***Effect of physico-chemical parameters of water on the presence of the organism**:*

This study revealed a higher turbidity in river/stream, a higher degree of hardness in pond water as well as a higher resistivity, higher electrical conductivity and TDS in brackish waters and higher electrical conductivity in seawater, which are usually less conducive for the survival of the organism. An alkaline pH in lake water favoured the survival of the organism. The physicochemical parameters of the water from various sources are given in table 3.

The correlation of total viable count (TVC) and coliform count (CC) with the presence and absence of the organism

in river waters revealed that no significant difference existed between the presence of the organism and the TVC and CC. Analysis of well water revealed that the physico-chemical parameters had no significant effect on the presence of the organism. The organism was found to be absent in brackish waters with a higher resistivity and also in waters with higher electrical conductivity and total dissolved solids.

On analysis of the effect of the various parameters on the presence of the organism in seawater, it was observed that only conductivity had a significant effect on the presence of the organism in the water. A higher electrical conductivity was not favourable for the organism. In the deep sea-water samples, the salinity was on the higher side, and the organism has been known to tolerate up to two per cent salinity only. Shore or coastal water samples had lesser salinity due to the influx from the freshwater bodies. Except for Chavakkad harbor, there was a whole lot of activity like washing of boats, fish containers, harbor floor and dumping fish waste into the coastal waters, which may have contributed to the presence of the organism in the coastal water.

Campylobacter spp. is ubiquitous in the environment, with an array of reservoirs or susceptible hosts. No significant correlation between indicator organisms and the presence of campylobacter could be observed, which is in agreement with the finding of Carter *et al.* (1987). Campylobacter concentrations had a low significant correlation with both *E. coli* and river flow only at the Grand River north location (Van-Dyke *et al.,* 2010). Therefore, water quality parameters (physico-chemical and microbiological) are not of much use in predicting campylobacter occurrence or it's concentrations in surface water samples.

CONCLUSION

Campylobacter, having emerged as one among the four important foodborne pathogens has raised public health concern worldwide, since a considerable number of acute bacterial enteritis in the Western world is being attributed to these organisms. The study points to the increasing occurrence of Campylobacter in water sources in central Kerala, and the public health significance of the organism. Hence, a comprehensive strategic approach could be devised to check the emerging role of this pathogen in foodborne diseases in the state

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COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Carter, A.M., Pacha, R.E., Clark, G.W. and Williams, E.A. 1987. Seasonal occurrence of *Campylobacter* spp. in surface waters and their correlation with standard indicator bacteria. *Appl. Environ. Microbiol*. **53**(3): 523-526.

Chon, J., Hyeon, J., Yim, J., Kim J., Song, K. and Seo K. 2012. Improvement of Modified Charcoal-Cefoperazone-Deoxycholate agar by supplementation with a high concentration of Polymyxin B for detection of *Campylobacter jejuni* and *C. coli* in chicken carcass rinses. *Appl. Environ. Microbiol*. **78** (5): 1624-1626.

Daczkowska-Kozon, E. and Brzostek-Nowakowska,J.2001.*Campylobacter* spp. in waters of three main western Pomerania water bodies. *Int. J. Hyg. Environ. Hlth.* **203**(5-6): 435-43.

Denis, M., Soumet, C., Rivoal, K., Ermel, G., Blivet, D., Salvat, G., Colin, P. 1999. Development of m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. *Lett. Appl. Microbiol.* **29**(6):406-10.

Ferrari, S., Frosth, S., Svensson, L., Fernstrom, L.L., Skarin, H. and Hansson, I. 2019. Detection of *Campylobacter* spp. in water by dead-end ultrafiltration and application at farm level. *J. Appl. Microbiol*. **127**(4): 1270-1279.

Ghane, M., Moein, F. G. and Massoudian, S. 2012. The first isolation of *Campylobacter jejuni* from Caspian sea's water. *Advanced Stud. Biol.* **4**(9): 407–418.

Hakeem, M.J. and Lu, X. 2021. Survival and control of Campylobacter in poultry production environment. *Front. Cellular Infect. Microbiol.* **10**: 904.

Horman, A., Rimhanen-Finne, R., Maunula, L., Bonsdorff, C.H., Torvela, N., Heikinheimo, A. and Hanninen, M.L. 2004. *Campylobacter* spp., *Giardia* spp., *Cryptosporidium* spp., Noroviruses, and indicator organisms in surface water in southwestern Finland. 2000-2001. *Appl. Environ. Microbiol.* **70**(1): 87-95.

Igwaran, A. and Okoh, A.I. 2019. Human campylobacteriosis: A public health concern of global importance. *Heliyon.* **5**: 1-14.

Jones, K. 2001. Campylobacters in water, sewage and the environment. *Symp. Ser. Soc. Appl. Microbiol.* **30**: 68.

Kemp, R., Leatherbarrow, A.J., Williams,

N.J., Hart, C.A., Clough, H.E., Turner, J., Wright, E.J., and French, N.P. 2005. Prevalence and genetic diversity of *Campylobacter* spp. in environmental water samples from a 100-square-kilometer predominantly dairy farming area. *Appl. Environ. Microbiol*. **71**(4): 1876–1882.

Khan, I. and Edge, T.A. 2013. Investigation of the prevalence of thermophilic *Campylobacter* species at Lake Simcoe recreational beaches. *Inland Waters*. **3**: 93–104.

KSCSTE. 2021. Climate. ENVIS Centre: Kerala State of Environment and Related Issues. Kerala State Council for Science, Technology and Environment.

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

OIE. World Organisation for Animal Health. Terrestrial Manual. 2017. Chap. 2.9.3. *Campylobacter jejuni* and *Campylobacter coli*. pp. 1185-1191.

Moore, J., Caldwell, P. and Millar, B. 2001. Molecular detection of *Campylobacter* spp. in drinking, recreational and environmental water supplies. *Int. J. Hyg. Environ. Hlth*. **204**(2-3): 185-189.

Pitkänen, T. 2013. Review of *Campylobacter* spp. in drinking and environmental waters. *J. Microbiol. Methods*. ***95*:** 39–47.

Pitkanen, T. and Hanninen, M. L. 2017. Members of the family Campylobacteraceae: *Campylobacter jejuni*, *Campylobacter coli*. In: J.B. Rose and B. Jiménez-Cisneros, (eds) Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project). (A. Pruden, N. Ashbolt and J. Miller (eds), Part 3: Specific Excreted Pathogens: Environmental and Epidemiology Aspects - Section 2: Bacteria), Michigan State University, E. Lansing, MI, UNESCO. https://doi.org/10.14321/waterpathogens.23

Popowski, J., Lekowska-Kochaniak, A.and Korsak, D. 1997. The incidence of heat tolerant Campylobacter in rivers and lakes of the Warsaw region. *Rocz. Panstw. Zakl. Hig*. **48**(3): 253-62.

Rollins, D.M. and Colwell, R.R. 1986. Viable but nonculturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. *Appl. Environ. Microbiol.* **52**(3): 531–8.

Rozynek, E., Dzierzanowska-Fangrat K., Jozwiak, P., Popowski, J., Korsak, D. and Dzierzanowska, D. 2005. Prevalence of potential virulence markers in Polish *Campylobacter jejuni* and *Campylobacter coli* isolates obtained from hospitalized children and from chicken carcasses. *J. Med. Microbiol*. **54**: 615-619.

Savill, M.G., Hudson, J.A., Ball, A., Klena, J.D., Scholes, P., Whyte, R.J., McCormick, R.E. and Jankovic, D. 2001. Enumeration of Campylobacter in New Zealand recreational and drinking waters. *J. Appl. Microbiol.* **91**: 38–46.

Sharma, B., Parul, S.,Verma A.K., Jain, U., Mishra, R. Yadav J.K. and Singh R. 2016. Project on Quality analysis of drinking and Yamuna water from different areas of Mathura and Agra regions with special reference to *E. coli* and Campylobacter.pp.155.

Srigowthami, P. 2013. Studies on the incidence of *Campylobacter jejuni* in livestock products and environmental samples. *M.V.Sc. Thesis*, SriVenkateswara Veterinary University Rajendranagar, Hyderabad. 122p.

Stern, N.J., Line, J.E. and Chen, H.C. 2001. *Campylobacter*. In: Downes, F.P. and Ito, K. (eds.), *Compendium of Methods for the Microbiological Examination of Foods*. (4th Ed.). American Public Health Association, Washington, D. C. pp. 301–310.

Szczepanska, B., Andrzejewska, M., Spica, D. and Klawe, J.J. 2017. Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from children and environmental sources in urban and suburban areas. *BMC Microbiol.***17**: 80.

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, 101p.

Whiley, H., van-den-Akker, B., Giglio, S. and Bentham, R. 2013. The role of environmental reservoirs in human campylobacteriosis. *Int. J. Environ. Res. Public Hlth*. **10**(11): 5886–5907.

Yaman, H. Elmali, M., Ulukanli, Z., Atabay, H. and Tekinşen, K. 2005. Presence of Campylobacter (*C. jejuni*) in recreational, lake and stream water and fresh fish in Turkey. *Archivfür Lebensmittelhyg.* **56**(4): 83-86.