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Occurrence of thermophilic *Campylobacter* spp. in pigs and the assessment of biosecurity measures employed at unorganized pig farms in Thrissur, Kerala

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Abstract

Campylobacter spp. is considered as one of the major causes of foodborne illnesses worldwide. A total of 130 samples including faecal samples (n=40), rectal swabs (n=40) and sewage samples (n=50) were collected from the two unorganized pig farms to study the occurrence of Campylobacter spp. The biosecurity measures on the farms were also assessed. An overall occurrence of 26.15 per cent with a higher rate of isolation from rectal swabs (57.5per cent) than faecal and sewage samples (25 per cent and 2 per cent) were observed. The occurrence of C. coli was found to be 55per cent, while that of C. jejuni and C. coli was 5per cent in rectal swabs collected from Farm A. Campylobacter coli could be isolated only from the sewage sample from farm B. Direct multiplex PCR screening detected C. coli in 32per cent and 44per cent of sewage samples from farms A and B, respectively. This indicates that the Campylobacter organisms in sewage samples might have attained viable but not culturable form. In both farms, no effective biosecurity measures were followed. The lack of biosecurity measures in farms contributes to the transmission of Campylobacter spp. from the environment to the animals. Farm workers of both the farms were unaware of hygienic practices and biosecurity measures. Furthermore, little attention was paid to personal protective measures, which could pose a significant occupational risk of contracting campylobacteriosis, resulting in complex sequelae.

Keywords: *Biosecurity, Campylobacter, Campylobacteriosis, One Health, Pig farm*

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Campylobacter spp. are often considered to be one of the leading causes of foodborne illnesses in humans and are implicated in approximately 400 to 500 million diarrheal diseases (Ruiz-Palacios, 2007) with serious complications such as Guillain-Barre Syndrome, Miller Fisher Syndrome, Irritable Bowel Syndrome, and Reactive Arthritis (Smith, 2002; Peterson, 1994). Direct contact with farm animals, consumption of raw meat, and handling of contaminated food items are the major risk factors for campylobacteriosis in humans (Kuhn *et al.,* 2017). Campylobacteriosis is a major public health problem with complex epidemiology (Humphrey *et al.,* 2007) and chicken and pigs are the main reservoirs and primary sources of *Campylobacter jejuni* and *Campylobacter coli* infections, respectively in humans. Furthermore, the absence of biosecurity measures on pig farms exposes them to the reservoirs of infection leading to the colonisation of *Campylobacter* in pigs (Brown *et al.,* 2004). To avoid organism contamination and multiplication, strict biosecurity procedures are essential (Athulya *et al.,* 2021). Campylobacteriosis remains a bacterial foodborne pathogen that will pose a significant threat to public health in the coming years (Deepa *et al.,* 2022). Hence, the possible risk of campylobacteriosis in pigs and the entry of *Campylobacter* spp. into the food chain should be viewed seriously. Hence, the present study was conducted to assess the occurrence of *Campylobacter* spp. infection in pigs and biosecurity measures observed if any in two pig farms in Thrissur district, Kerala.

Materials and methods

Sample processing and molecular confirmation

A total of 130 samples including rectal swabs, faecal and sewage samples were collected from September to December, 2019 from two unorganized pig farms in Thrissur, (Table 1). The samples were collected from pigs aged between one to six months. All the samples were transported to the laboratory in a cold chain and immediately processed as per the OIE (2017) guidelines. Rectal and faecal samples were directly streaked on modified charcoal cefoperazone deoxycholate agar (mCCDA) plates, while sewage samples were enriched on charcoal cefoperazone deoxycholate (CCD) broth and incubated at 42°C in a carbon dioxide (CO_2) incubator with

Table 1. Details of samples collected from pig farms

Sample Sources	Pig Farms		Total
	Pig Farm A	Pig Farm B	
Rectal Swabs	20	20	40
Faecal sample	20	20	40
Sewage sample	25	25	50
Total	65	65	130

Table 2. Details of primers and product size

Gene	Primer sequence	Annealing Temperature	Size (bp)	Reference
16S rRNA	F – 5’-GGATGACACTTTTCGGAGC-3’	51.8 ⍰	816	Linton *et al*. (1996)
	R - 5’-CATTGTAGCACGTGTGTC-3’			
*map*A	F - 5’-CTATTTTATTTTTGAGTGCTT GTG-3’		589	Denis *et al.* (1999)
	R - 5’-GCTTTATTTGCCATTTGTTTT ATTA-3’			
*ceu*E	F - 5’-AATTGAAAATTGCTCCAAC TATG-3’		462	Denis *et al.* (1999)
	R - 5’-TGATTTTATTATTTGTAGC AGCG-3’			

ten per cent CO_2 for 48 hours before streaking on mCCDA plates. Genomic DNA extracted from enriched sewage samples by snap chill method was used for confirming the isolates by performing a multiplex polymerase chain reaction (mPCR) (Englen and Kelley, 2000) targeting the *16S* rRNA gene for *Campylobacter* genus, *map*A gene for *C. jejuni* and *ceu*E gene for *C. coli*. Information on primers, annealing temperature and product sizes are shown in Table 2. The *C. jejuni* (NCTC 11168) and *C. coli* (NCBI accession no: OM810312) were used as positive controls in mPCR. Physico-chemical parameters such as pH, dissolved oxygen (DO), total dissolved solids (TDS), conductivity, temperature, and salinity and biochemical oxygen demand (BOD) of sewage samples collected from both the farms were analysed (APHA, 2005).

. Knowledge about personal hygiene measures and farm biosecurity measures among pig farm workers were analysed. A Chi-square test using SPSS version 24.0 software was employed to analyse the difference in the occurrence of *Campylobacter* spp. between farms and between different sources of samples.

Assessment of biosecurity measures in pig farms

Biosecurity measures such as fencing, over nets, flies and rodent traps, disinfectant dips for vehicles and workers, water source accessibility to scavenging birds, feeding trough, feed and feeding practices, movement of pet/stray animals inside farms, visitor's records and farm worker's hygienic practices were analysed in both the farms to determine the possibilities of transmission of *Campylobacter* spp. from the environmental reservoirs to the pigs.

Results and discussion

On molecular confirmation of *Campylobacter* genus and species by mPCR assay, all 34 isolates yielded an amplicon of 816 bp size, specific for *Campylobacter* genus. An amplicon of 462 bp size, specific for *C. coli* was obtained with 94.12per cent of isolates while, 2.94per cent of isolates generated an amplicon of 589 bp size, specific for *C. jejuni*. 2.94 per cent of the isolates yielded amplicons of 462 bp and 589 bp (combination of *C. jejuni* and *C. coli*) (Fig. 1).

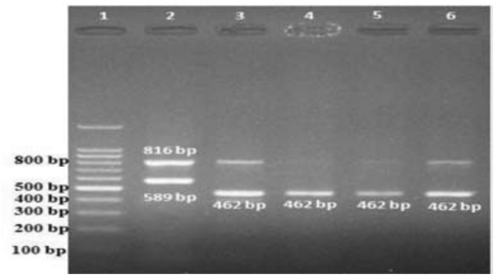

Fig.1 Amplicons of mPCR

Genus: *16S* rRNA (816 bp)
Lane 1: 100 bp plus ladder
Lane 2: *C. jejuni* positive control (589 bp)
Lane 3: *C. coli* positive control (462 bp)
Lane 4-6: Field isolates *C. coli* (462 bp)

The occurrence of *Campylobacter* spp. in the pig farms was 26.15 per cent and was comparable to the findings of Muralikrishna (2018) and Karikari *et al*. (2017), who reported an overall occurrence of 27.5 per cent and 28.7 per cent from pig farms in Kerala and Ghana, respectively. However, no significant difference was observed in the occurrence of *Campylobacter* in these two farms. The occurrence of *C. coli* observed in this study (57.5 per cent) is consistent with the findings of Gebreyes *et al*. (2005), who found that 55.8 per cent of pigs in the United States harboured *C. coli.* The rate of isolation of *C. coli* from rectal swabs was significantly higher ($p < 0.001$) compared to the isolation rate of *C. jejuni* and/or a combination of *C. jejuni* and *C. coli* from both farms. Moreover, the rate of isolation of *Campylobacter* spp. from rectal swabs was comparatively higher than those from faecal samples. In this context, it is worth noting that *C. coli* is a human pathogen more commonly implicated in indigenously acquired foodborne illnesses than *Salmonella enterica* serovar Typhimurium (Tam *et al.,* 2003). Occurrence of *Campylobacter* spp. from different sources of both the farms are represented in Table 3.

Table 3. Details of *Campylobacter* spp. isolates from different sources

	***Campylobacter* spp.**	**Occurrence & Source**			**Overall occurrence**
		Rectal swabs	**Faecal samples**	**Sewage samples**	
Farm (A)	*C. jejuni*	0	5	0	1.53
	C. coli	55	20	0	23.07
	Combination of *C. jejuni* & *C. coli*	5	0	0	1.53
	Occurrence	60^{a}	25	0	26.15^{ns}
Farm (B)	*C. jejuni*	0	0	0	0
	C. coli	55	25	4	26.15
	Combination of *C. jejuni* & *C. coli*	0	0	0	0
	Occurrence	55^{a}	25	4^{b}	26.15^{ns}
Overall occurrence		57.5	25	2	26.15

$^{a, b}$ significant at 0.01 level; ^{ns} not significant

Screening of samples from farms A and B by mPCR revealed the presence of the DNA of *C. coli* in 32per cent and 44per cent of the samples respectively. However, *C. coli* could be cultured only from 4per cent of sewage samples from the farm B (Table 3). In this study, the direct screening of enriched sewage samples by mPCR detected *C. coli* in 32 per cent and 44 per cent on farm A and B, respectively. *Campylobacter* might be difficult to isolate in environmental samples (Dyke *et al.,* 2010). This might be due to poor cell recovery using standard selective culture techniques, low concentrations of often damaged or stressed cells, and the development of viable but non-culturable cells (VBNC) (Dyke *et al.,* 2010). When *Campylobacter* is found outside the gastrointestinal tract and exposed to the environment, it quickly develops into VBNC forms (Rollins and Colwell, 1986). Although VBNC form of *Campylobacter* spp. are not easily discovered using standard culture techniques, their pathogenicity is equivalent to that of culturable forms (Thomas *et al.,* 1998).

The physico-chemical parameters of the sewage samples from both the farms were within normal limits, except for BOD_5 (Table 4), which was much higher than the maximum permitted level of 30 mg/L according to Indian Standards for Quality of Effluents IS:10500, Part A (CPCB, 2019) and can lead to environmental pollution around farms if discharged untreated.

Effective biosecurity measures such as disinfection dips, fencing to restrict visitor's entry, and measures to deter/control scavenging birds, pests and rodents were absent on both the farms (Table 5; fig. 2), which led to the easy accessibility of feeding troughs and sources of drinking water to cranes and crows. Crow mobbing in farm settings (Houston, 1977) and subsequent contamination of the farm environment with their faeces may lead to cross-transmission of *Campylobacter* spp. among animals and occupational groups (Muralikrishna *et al.,* 2018). Effective implementation of biosecurity measures is vital in controlling campylobacteriosis in pig farms. Feeding of raw chicken waste observed on both the farms can be a potential source of *Campylobacter* spp. infection and throws light on the importance of feeding cooked chicken waste, as a majority of caecal samples taken from commercial and backyard poultry settings revealed the presence of *C. coli* (Rangaraju *et al.,* 2022).

Table 4. Physico-chemical parameters of sewage samples

S.No.	**Parameters**	**Farm A**	**Farm B**
1.	pH	7.104	7.753
2.	Salinity (PSU)	6.565	4.113
3.	TDS (ppt)	3.69	1.4
4.	DO (mg/L)	11.81	9.54
5.	Conductivity (µs/cm)	11.70	7.38
6.	BOD (mg/L)	900	800
7.	Temperature (□)	29.2	30.1
8.	Resistance Ω/cm	134.5	84.21

Farmworkers in both farms were not using personal protective equipment and were not aware of personal hygiene measures (Table 5). Farm owners were advised to give adequate

Table 5. Biosecurity measures followed in pig farms

S. No	Biosecurity Measures	Farm A	Farm B
1.	Fencing around farm	No	No
2.	Effective over nets	No	No
3.	Fly and rodent traps	No	No
4.	Disinfectant dips at farm and animal area entry	No	No
5.	Accessibility of water source and feeding trough to wild birds	Yes	Yes
6.	Movement of pet animals inside farm premises	Yes	No
7.	Visitor's record	No	No
Farm worker's hygienic practices/personnel protection			
1.	Wearing gloves	No	No
2.	Wearing gumboots	No	No
3.	Wearing overalls	No	No
4.	Washing hands and legs with soap before and after farm operations	No	No
5.	The habit of using hand sanitizers in farm premises	No	No

Fig. 2. Biosecurity breaches in pig farm: a) No fencing on the farm to prevent entry of unauthorized persons, stray and wild animals, b) No over nets to prevent the entry of wild birds c) No fly and rodent traps, d) Animal feeding and water trough were easily accessible to wild birds, e & f) Accessibility of animal area to wild birds such as crane and crow and g) Disinfection dips not installed at the entry points.

training to employees on personal hygiene and implement strict biosecurity measures to control the occurrence of campylobacteriosis in pigs.

Conclusion

Campylobacter spp. is one of the leading causes of foodborne gastroenteritis worldwide. The present study pointed out the predominance of C. coli on both the farms and lack of sewage treatment plant, biosecurity measures and personal protection equipments for pig farm workers. It is therefore advised to establish sewage treatment plants and adequate biosafety measures in pig farms and conduct awareness programmes for farm workers to reduce the spread of Campylobacter spp. among animals and subsequent spread of infection to humans through a holistic One Health approach.

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Conflict of interest

The authors declare no conflict of interest.

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