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Occurrence of *Campylobacter* spp. in a pork processing line in Thrissur, Kerala

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Abstract
Campylobacteriosis was estimated to cause 400 to 500 million cases of human diarrhoea every year worldwide. Poultry and pigs are the major reservoirs. The risk of *Campylobacter* infection through processing of meat needs to be investigated carefully as large quantities of pork meat are consumed around the globe. Samples were collected from a pork processing line in Thrissur, Kerala, to determine the occurrence of *Campylobacter* spp. Eighty samples were collected consisting of carcass swabs (n=20), knife swabs (n=20), cutting board surfaces (n=20) and sewage samples (n=20). Campylobacter isolates were circular, flat to slightly elevated, grey in colour with spreading tendency on modified charcoal cefoperazone deoxycholate agar plates. The virulence gene *cad* F was harboured by all the isolates in this study. These genes play critical roles in *Campylobacter* adherence and colonisation of human intestinal epithelial cells during infection in humans. Carcass swabs of ham region contained 25 percent of *C. coli* per 100 sq. cm of surface area. *Campylobacter* was not found in the other two carcass points, namely the belly and jowl region. *Campylobacters* could not be isolated from the liquid effluent. This might be due to low concentrations of often injured or stressed cells, the formation of viable but non-culturable cells, and poor cell recovery using conventional selective culture methods. Occurrence of *C. coli* was observed as 10 and 15 percent from knife and cutting board surfaces respectively. The presence of *Campylobacter*s in the slaughter house environment is cause for concern since these sources can enhance the likelihood of cross-contamination across carcasses. Improved hygiene management on worker's standard operating procedures and slaughtering equipment might lower *Campylobacter* pathogen levels in pork processing plants. A holistic One Health approach is essential to eliminate the transmission of *Campylobacters* in animals and humans.

Keywords: *Campylobacter*, pork processing line, carcass, knife, cutting board

Introduction
Campylobacteriosis was estimated to cause 400 to 500 million cases of human diarrhoea every year worldwide, although this infection is sporadic and self-limiting. A low infection dose of about 500 cells can derive the initial clinical symptoms. Campylobacteriosis is characterized by watery and sometimes bloody diarrhea, fever, abdominal cramps, and vomiting (Ruiz-Palacios, 2007; Korsak, 2014; Skarp *et al.*, 2016; Zang *et al.*, 2018) [32, 18, 33, 35]. Eighty to ninety percent of the *C. jejuni* and five to ten percent of *C. coli* infections in humans are associated with the consumption of contaminated meat (Biasi *et al.*, 2011) [4]. In European countries, pork meat remains a primary source for transmission of *Campylobacter* spp. to humans (Ghafir *et al.*, 2008) [9]. Although *Campylobacter* spp. contamination in animal origin food has been reported in several studies, most researches were focused on poultry and poultry products. The source of *Campylobacter* contamination on pig carcass is not clear, although pig is also considered as potential reservoir of *Campylobacter* spp. (Alban *et al.*, 2008) [1]. In the farms, *Campylobacter* spp. can colonise the gastrointestinal tract of pigs and pass out in its faeces. *Campylobacter coli* isolates are often recovered from pig faeces (Stella *et al.*, 2017) [34]. During slaughter and subsequent steps, *Campylobacter* spp. might spread in slaughtering environment and slaughtering operations, and finally contaminate the food chain (Hansson *et al.*, 2007; Hermans *et al.*, 2012) [11,13]. The risk of *Campylobacter* infection through processing of meat needs to be investigated carefully as large quantities of pork are consumed around the globe (Nesbakken *et al.*, 2003) [25]. However, the epidemiological data on *Campylobacter* spp. infection in Asia is still limited, and incidences reported by other countries vary substantially (Kaakoush *et al.*, 2015) [16]. Hence, this study was undertaken to study the occurrence of *Campylobacter* spp. in a pork processing line in Thrissur, Kerala.

Materials and Methods

Samples were collected from a pork processing line in Thrissur, Kerala, to determine the occurrence of *Campylobacter* spp. During the slaughter process, 80 samples were collected. Sample consisting of 20 carcass swabs were collected using sterile cotton swabs with a help of metal frame template size of 10 cm × 10 cm from three points on the carcass, namely the jowl, belly, and ham. Twenty swab samples each from knife and cutting board surfaces were collected from the processing plant. Swabs were dipped in seven millilitres of Cary Blair (Hi Media, India) medium containing three percent charcoal in a screw-capped tube. Twenty samples of liquid waste produced during pork processing operations were also collected from the processing plant.

Samples were transported immediately to the laboratory of Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Mannuthy, Thrissur in cold chain. Samples were processed within 4h by enriching the samples in modified charcoal cefoperazone deoxycholate broth and incubated under 10 percent carbon dioxide (CO_2) at 42 °C for 48 hours (h) followed by streaking a loopful of enriched samples on modified charcoal cefoperazone deoxycholate agar (mCCDA) (Hi Media, India) plates supplemented with CAT (Cefoperazone, Amphotericin B and Teicoplanin) selective supplement (FD 145), *Campylobacter* supplement V (FD 067) and Polymyxin B selective supplement (FD 003) and incubated under 10 percent CO_2 condition at 42 °C for 48 h for isolation of *Campylobacter* spp. according the protocol given by the OIE terrestrial manual (OIE, 2017) [26].

The genomic DNA of *Campylobacter* spp. suspected colonies were isolated by snap chill method (Englen and Kelley, 2000) [8] and multiplex polymerase chain reaction (mPCR) was performed on an automated thermal cycler (Eppendorf Master Cycler, Germany) for molecular confirmation of *Campylobacter* genus by targeting the *16S* rRNA gene (Primers: (Forward): 5'-GGATGACACTTTTCGGAGC-3', (Reverse): 5'-CATTGTAGCACGTGTGTC-3') (Linton *et al.*, 1996) [20], for *Campylobacter jejuni* (*C. jejuni*) by targeting *map*A (Primers: (Forward): 5'-CTATTTTATTTTTGAGTGCTTGTG-3', (Reverse): 5'-GCTTTATTTGCCATTTGTTTTATTA -3') (Denis *et al.*, 1999) [5], for *Campylobacter coli* (*C. coli*) by targeting *ceu*E (Primers: (Forward): 5'-AATTGAAAATTGCTCCAACTATG-3', (Reverse): 5'-TGATTTTATTATTTGTAGCAGCG -3') (Denis *et al.*, 1999) [5], and virulence gene *cad*F (Primers: (Forward): 5'-TTGAAGGTAATTTAGATATG-3', (Reverse): 5'-CTAATACCTAAAGTTGAAAC-3') (Bang *et al.*, 2003) [2] with an annealing temperature of 51.8 °C.

Genomic DNA of NCTC 11168 (*C. jejuni*) and *C. coli*, accession no: OM810312 were used as positive control. Amplified PCR product (5 µL) was mixed with gel loading dye containing bromothymol blue and the samples were loaded into the wells with 100 bp plus DNA ladder as DNA molecular size marker, in order to compare the size of the amplified product. The gel was visualised and the images were documented on gel documentation system (Syngene, USA).

Results and Discussion

Among 80 samples processed, an overall *Campylobacter* spp. occurrence of 12.5 percent was observed. These isolates were circular, flat to slightly elevated, grey in colour with spreading tendency on mCCDA plates (Fig 1), in accordance with OIE, (2017) [26]. This charcoal-based media might be utilised successfully without the need for live animal blood. This was in agreement with the findings of Engberg *et al.* (2000) [7] and Oyarzabal *et al.* (2005) [28], who described mCCDA as an efficient medium with improved isolation rates. On Gram staining, all of the isolates were Gram negative, spirally rod or sea-gull shaped (Fig 2), oxidase and catalase positive (Fig 3 and 4) and hippurate hydrolysis negative (Fig. 5). These findings were consistent with those of Muralikrishna (2018) [23] and Jolly (2021) [15] who found identical *Campylobacter* characteristics in terms of Gram staining and biochemical activity. The mPCR revealed that all of the isolates were *C. coli* (Fig. 6). Kempf *et al.* (2017) [17] also isolated *C. coli* from all slaughter house samples. All the isolates in this study harboured the virulence gene *cad*F. These genes play critical roles in *Campylobacter* adherence and colonisation of human intestinal epithelial cells during infection in humans (Ghorbanalizadgan *et al.*, 2014) [10]. The presence of the highly conserved *cad* F virulence gene in all *Campylobacter* spp. was also reported by Muralikrishna *et al.* (2018) [24] and Barakat *et al.* (2020) [3] in their studies.

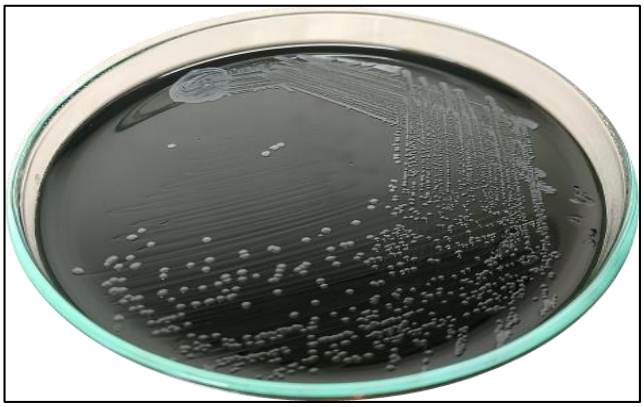


Fig 1: *Campylobacter* spp. colonies on mCCDA agar plates

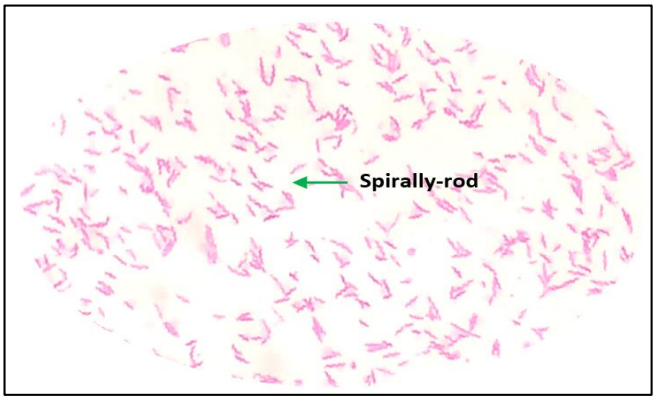


Fig 2: Grams staining

Fig 3: Oxidase Test

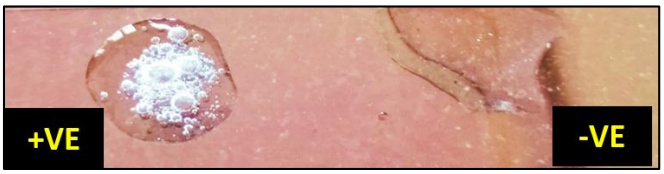


Fig 4: Catalase test

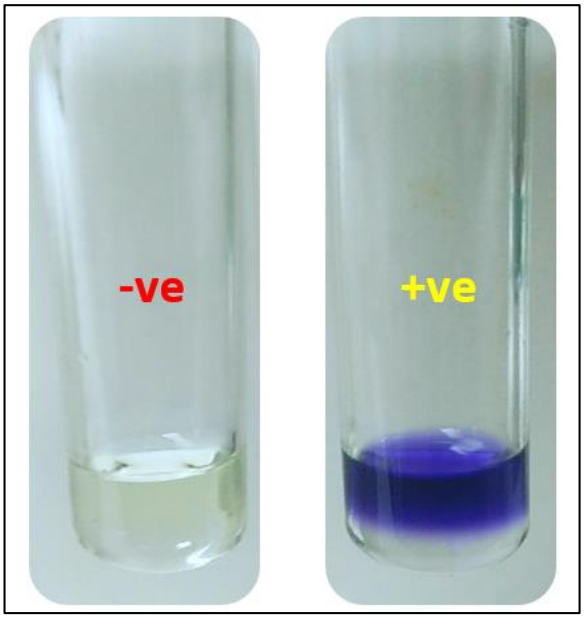
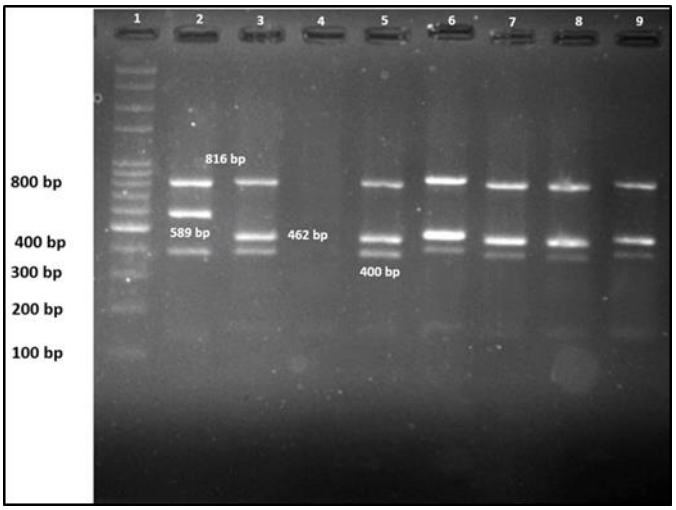


Fig 5: Hippurate hydrolysis test



Genus: *16s* rRNA (816 bp)
Virulence gene (*cad*F): 400 bp
Lane 1: 100 bp plus ladder
Lane 2: *C. jejuni* (NCTC 11168) positive control (589 bp)
Lane 3: *C. coli* positive control (462 bp) (Accession no: OM810312)
Lane 4: Negative control
Lane 5-9: Field isolates *C. coli* (462 bp)

Fig 6: mPCR image of *Campylobacter* spp. isolates

Carcass swabs of ham region contained 25 percent of *C. coli* per 100 sq. cm of surface area. Prevalence was found to on the higher side than the reports of Kwiatek *et al.* (1990) [19]; Meng and Doyle (1998) [22]; Harvey *et al.* (1999) [12]; Madden *et al.* (2000) [21]; Nesbakken *et al.* (2003) [25]; Pezzotti *et al.* (2003) [30], reported up to 10.3 percent *Campylobacter* prevalence from pork carcasses. The presence of *Campylobacter*s in the slaughterhouse environment is cause for concern since these sources can enhance the likelihood of cross-contamination across carcasses (Rangaraju *et al.*, 2022). *Campylobacter* was not found in the other two carcass points, namely the belly and jowl region. *Campylobacters* could not be isolated from the liquid effluent of the slaughterhouse. *Campylobacter* might be difficult to isolate in environmental samples (Dyke *et al.*, 2010) [6]. This might be due to low concentrations of often injured or stressed cells, the formation of viable but non-culturable cells, and poor cell recovery using conventional selective culture methods (Dyke *et al.*, 2010) [6].

In this study, samples collected from the surface of the knife were positive (10 percent) for *Campylobacter coli*. It is well known that a pork carcass can easily be contaminated with intestinal waste during slaughtering, and the knives are likely to be contaminated with *Campylobacter* spp. from the intestinal waste, especially in the process of evisceration. Although the slaughtering knives are periodically sterilised during slaughter operations, there is always a risk of incomplete disinfection and operational errors. Cutting boards also harboured 15 percent of *Campylobacter coli*. Muralikrishna (2018) [23] reported 10 percent prevalence from knife and cutting board samples of pork processing line. Oosterom *et al.* (1985) [27], ICMSF, (1998) [14] and Pearce *et al.* (2003) [29] considered slaughtering equipment to be a significant risk factor for *Campylobacter* cross-contamination in pork.

Conclusion

Campylobacter coli was prominent in all samples from the slaughterhouse environment. Pork carcasses had a higher rate of occurrence. Slaughterhouse personnel must be trained on food safety standards and hygienic carcass handling. Slaughtering operators should also be made aware that the knife and cutting board surfaces be regarded as a risk element. Improved worker and equipment hygiene management might lower *Campylobacter* pathogen levels in pork processing plants. To eliminate *Campylobacter*, novel approaches such as the use of phytochemicals, plant extracts and bacteriophages on food contact surfaces are crucial from food safety point of view. A holistic One Health approach is essential to eliminate the transmission of *Campylobacters* in animals and humans.

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Conflict of interest

The authors declare no conflict of interest.

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