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Occurrence of thermophilic *Campylobacter* in an organised cattle farm in kerala, India

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Abstract
Foodborne diseases comprise a broad spectrum of illnesses and an escalating public health problem worldwide. Foods of animal origin and drinking water are commonly considered as the key source of food borne infections. *Campylobacter* spp. are important zoonotic bacterial pathogens which mainly cause foodborne enteritis in humans. The organism is generally dispersed across a wide range of animals, including livestock, poultry, and wildlife, and is transmitted to human beings mainly by the consumption of contaminated food, water, and milk. In this study the occurrence of thermophilic campylobacter in an organised farm in Kerala was evaluated. The overall prevalence rate of thermophilic *Campylobacter* was found to be 16 per cent by multiplex polymerase chain reaction with dung (four per cent), milk (four per cent), water (four per cent) and soil samples were found to harbour *Campylobacter* spp. Two *Campylobacter coli* isolates were obtained from the faecal samples and the isolates were sensitive to amikacin, amoxycillin, chloramphenicol, ciprofloxacin, clindamycin, dorepenem, doxycycline, gentamicin, imipenem and meropenem and resistant to cefixime, cefotaxime, cefuroxime, ceftazidime, enrofloxacin and tetracycline.

Keywords: Foodborne diseases, *Campylobacter spp*., polymerase chain reaction, kerala

Introduction
Foodborne infection is an important public health problem in world. Among the food borne pathogens, public health burden of *Campylobacter* has risen substantially all over world during the past 30 years. The food may get contaminated at any stage of processing, from production to consumption and can also result from environmental contaminations. It is estimated that, each year nearly 600 million people are affected by foodborne illness. *Campylobacter* spp. is recognised as one of the four major universal causes of diarrhoeal diseases. Foods of animal origin and drinking water are commonly considered as the key source of foodborne infection (Sczepanska, 2017) [1]. Rapid industrialization and urbanisation have resulted in the global expansion of production and consumption of animal products especially foods of animal origin due to its high nutritive value. India occupies fifth position in meat production.
The organism is generally dispersed across a wide range of animals, including livestock, poultry, and wildlife, and is transmitted to human beings mainly by the consumption of contaminated food, water, and milk (Tang, 2017) [2]. the organisms are excreted into the environment through faecal runoff of animals and birds, contaminating the surface water. They may also be transmitted during slaughter via meat surfaces by faecal contamination and infection may occur from improperly cooked meat and cross-contamination of uncooked food items. Consumption of beef or raw milk is the major cause of campylobacteriosis in humans, while direct contact between individuals has no relevance (Dasti *et al*., 2010) [3]. Studies on pathogenesis reveal that, for this organism to cause disease, susceptibility of the host and relative virulence of the infecting strain are both detrimental at an infective dose as low as 500 organisms.

Materials and methods
The present study was undertaken to study the occurrence of thermophilic *Campylobacter* in cattle and to understand the role of cattle in transmission of the organism to human beings.

Sample collection
A total of 175 samples including rectal swabs, water, milk and soil were collected from an organised cattle farm in Kerala from November 2017 to February 2018. All the samples were

Collected in sterile containers except rectal swabs, which were collected using sterile cotton swabs (Himedia, Mumbai) dipped in Cary Blair media. All the aseptically collected samples were transported at 4°C to the laboratory and were processed within four hours to ensure the viability of the organism.

Processing of samples

All the collected samples except rectal swabs were subjected to isolation and identification of *Campylobacter* spp. as per OIE (2017) [4]. with certain modifications. The rectal swabs were directly plated on mCCDA agar without enrichment.

Prior to enrichment, hundred millilitres of each of the water samples were subjected to membrane filtration through cellulose ester filters of 0.45 µm pore size and 47 mm diameter. The filters were then completely immersed in 225 ml of mCCD enrichment broth. In case of milk samples, fifteen millilitres of milk were centrifuged at 14,000 rpm for 20 minutes at 4^0C. The pellet was suspended in 45 ml of Blood Free *Campylobacter* (modified Charcoal Cefoperazone Deoxycholate, mCCD) broth (HiMedia, Mumbai) with CCDA selective supplement (FD 135) under microaerophilic conditions at 42°C for 48 hours.

Molecular detection

All the collected samples were directly subjected to multiplex polymerase chain reaction (m-PCR) targeting genus specific *16Sr*RNA region (816 bp), virulence gene *cad*F (400 bp), *C. jejuni* specific *map*A (589 bp) and *C. coli* specific *ceu*E (489 bp) genes, to detect *Campylobacter* spp. as per the procedure described by Denis *et al*. (1999) [6]. with slight modifications. In addition to this, all the isolates obtained by culture method were also subjected to m-P

Antibiotic resistance profiling

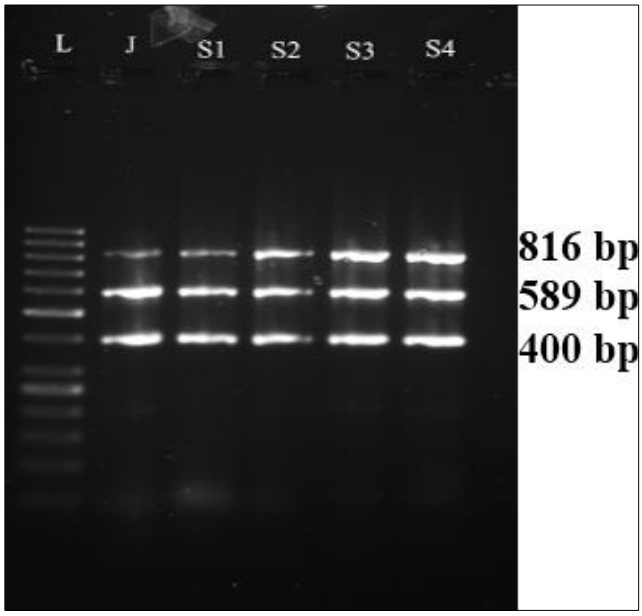
All the m-PCR positive isolates were subjected to antibiotic susceptibility test (ABST) by disc diffusion method. The sensitivity pattern was analysed using the disc diffusion assay, according to the criteria defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017) [7].

Result

Out of the 175 samples analysed, *Campylobacter* spp. were detected in 12 samples by direct multiplex PCR targeting genus specific *16Sr*RNA region (816 bp), virulence gene *cad*F (400 bp), *C. jejuni* specific *map*A (589 bp) and *C. coli* specific *ceu*E (489 bp) genes. It was found that, all the 12 samples had the virulence gene *cadF*, 10 samples were positive for *C. jejuni* specific *mapA* gene (Fig. 1) and two samples were positive for *C. coli* specific *ceu*E (Fig. 2.). *Campylobacter coli* was isolated from two of the rectal swabs collected from diarrhoeic calves which was already positive in direct m-PCR (Table 1). The two isolates were again subjected to m-PCR and positive amplicons were obtained in the genus specific *16Sr*RNA region, virulence gene *cadF* region and for *C. coli* specific *ceu*E region (Fig. 2). The amplicons were subjected to molecular sequencing and the sequences showed 100 per cent similarity to *Campylobacter coli* Indian isolate

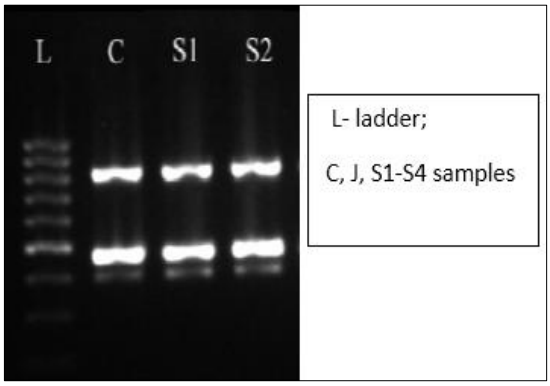
Table 1 Occurrence of *Campylobacter* spp. in an organised cattle farm

S. No	Source	Direct PCR		Culture	
		Total no. of samples	Positive (%)	Total no: of samples	Positive (%)
1	Rectal swab	50	8	50	4
2	Milk	55	7.27	55	0.00
3	Soil	30	0.00	30	0.00
4	Water	40	10	40	0.00
Total		175	16	175	1.14



Campylobacter jejuni

Fig 1: Detection of *Campylobacter jejuni* by multiplex PCR



Campylobacter coli

Fig 2: Detection of *Campylobacter coli* by multiplex PCR

Discussion

Campylobacter spp. are highly successful, opportunistic human pathogens, which has been identified as the leading cause of bacterial enteritis around the world. Among them, two thermotolerant species, *C. jejuni* and *C. coli*, are the main causes of the human disease. They are part of natural intestinal flora of a wide range of domestic and wild birds and animals and are environmentally ubiquitous requiring specific conditions for growth. Ruminants play a major role in the epidemiology of human *Campylobacter* infections via multiple transmission routes including direct contact (occupational exposure), consumption of unpasteurized milk and dairy products, and environmental contamination (e.g. Water and soil).

In the present study, the rate of occurrence of *Campylobacter* spp. in dung samples was found to be eight per cent, which is comparable with the findings of Chatur (2014) [8]. and Bailey *et al.* (2003) [9], who observed four per cent and six per cent prevalence rates for *Campylobacter* in cattle faeces, respectively. The difference in prevalence rate of thermophilic *Campylobacter* spp. may be attributed to isolation methods, herd size and type, geography, season, animal age, and number of animals investigated (Bae *et al*., 2005) [10]. The small genomic size along with high adenine-thiamine content in its DNA explains the fastidious nature of the organism and moreover lengthy lag phase made the organism a poor competitor with other gastrointestinal microbes which reduces its survivability in the gastrointestinal tract of animals.

Unpasteurized milk is a major cause of *Campylobacter* outbreaks (Kalman *et al*., 2000) [11]. An overall occurrence rate of 7.27 per cent was obtained in the present study for *Campylobacter* spp. in milk samples, which may be due to faecal contaminations in milk. The finding is in tune with that of Hussain *et al.* (2007) [12], who reported an overall prevalence of 10.2 per cent *Campylobacter* spp. in raw bulk milk samples in Pakistan. The low prevalence of *Campylobacter* spp. in raw milk samples is attributed to the antimicrobial activity of lactoperoxidase system in milk (Beumer *et al*.1988) [13]. The difference in prevalence rate may be due to the variations in hygienic measures adopted, detection techniques, climatic conditions and alterations in the temperature and level of oxygen to which they are exposed.

An overall prevalence rate of 10 per cent was recorded from water samples in this study, where 10 out of the 40 samples were detected to have *Campylobacter* spp. This is high when compared to the prevalence rate of 2.7 per cent, reported by Hu and Kuo (2011) [14] in Taiwan. However, the findings of the present study did not correlate with the results of Pearson *et al*. (1993), who detected *Campylobacter* spp. from 62 per cent of water samples using Indirect Fluorescent Antibody test. The low occurrence of organism in the present study can thus be attributed to the frequent chlorination of water sources and proper storage facilities. It was also reported that 99 per cent of *Campylobacter* get inactivated after 5 min of contact with 0.1 mg of free chlorine per litre (Blaser *et al*., 1986) [15].

The antibiotic resistance profile analysis of the isolates revealed cent per cent sensitivity to amikacin, amoxycillin, chloramphenicol, ciprofloxacin, clindamycin, dorepenem, doxycycline, gentamicin, imipenem and meropenem, while all the isolates were resistant to cefixime, cefotaxime, cefuroxime, ceftazidime, enrofloxacin and tetracycline. The resistance against multiple antibiotics detected in the current study was in accordance with the findings of Hong *et al*. (2007) [16].

Conclusion

An overall prevalence rate of 16 per cent from the farm was recorded in this study which emphasises the role of cattle and environmental factors in transmitting the disease to human beings. In humans, *C. jejuni* and *C. coli* are pathogens routinely causing acute diarrhoea, but conditions such as Guillain-Barré syndrome, reactive arthritis and post-infectious polyneuropathy leading to paralysis may also occur (Scpenzaka, 2017). Although the rigorous application of general biosecurity measures is likely to reduce herd infection and environmental contamination, sustaining such measures on the farm appears to be extremely difficult and needs to be supported by personnel hygiene and farm worker education and incentives. Control of *Campylobacter* spp. throughout the food chain requires implementation of food safety management systems based on well-established HACCP principles.

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