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Occurrence of thermophilic *Campylobacter* spp. in free-ranging chickens

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Abstract
Campylobacteriosis is one of the leading causes of foodborne bacterial gastroenteritis worldwide. An investigation was conducted to determine the occurrence of thermophilic *Campylobacter* spp. from free-ranging chickens. Chicken caecal samples were collected from fifty-four free-ranging chickens in Bareilly district, Uttar Pradesh. Samples were processed using modified charcoal cefoperazone deoxycholate agar (mCCDA) plates. They were then incubated at the microaerophilic conditions at 42 °C for 48 h and the presumptive colonies were subjected to Gram stain and other biochemical tests. Finally, suspected colonies were confirmed by targeting lipid gene *lpx*A performing a multiplex polymerase chain reaction. *Campylobacter coli* was isolated from three (5.55%) caecal samples and was confirmed with mPCR assay. It is highly essential to educate the free-ranging poultry rearers about the biosecurity measures, principles of food safety and hygienic handling of birds during rearing, slaughtering and cooking process to reduce the incidence of human campylobacteriosis.

Keywords: *Campylobacter*, chicken, free-ranging, food safety

Introduction
Campylobacter spp. is considered as a universal cause of foodborne gastroenteritis. *Campylobacter*s are motile, Gram-negative, corkscrew shaped, microaerophilic microorganisms. Improper biosecurity measures can lead to colonisation of *Campylobacter* spp. in the chicken intestine. Birds can colonise these organisms at an early age and results in rapid horizontal transmission (Battersby *et al.*, 2016) [3]. The two predominant *Campylobacter* species, *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*) can results in serious food safety issues (Adams and Moss, 2000) [1] in developing and transitional countries. Only a few *Campylobacter* cells from undercooked or raw poultry can cause human campylobacteriosis (Bhaduri and Cottrell, 2004) [4]. As estimated by the Centers for Disease Control and Prevention (CDC), *Campylobacter* is responsible for 1.3 million cases of foodborne illnesses in the USA annually (Scallan *et al.*, 2015) [15] and 4–5 hundred million diarrheal cases around the globe (Ruiz-Palacios, 2007) [14]. *Campylobacter* infection can result in serious sequelae such as Guillain-Barré syndrome (Scallan *et al.*, 2015) [15] and Miller-Fisher syndrome (Skarp *et al.*, 2016) [17]. Poultry meat includes meat from broilers, laying hens, duck and turkey (Epps *et al.*, 2013) [7] and their products cause about 60–80% of the global human campylobacteriosis (ECDC, 2015) [5]. Since campylobacteriosis is of utmost public health significance. This investigation was undertaken to determine the occurrence of thermophilic *Campylobacter* spp. from free-ranging chickens.

Materials and Methods
Sample processing and Genus identification
The chicken caecal samples (n = 54) were aseptically collected from the household free-ranging chickens from the villages in Bareilly district, Uttar Pradesh. All the samples were carried to the laboratory in cold chain and the samples were processed immediately within 4h by direct streaking of caecal scrapings on modified charcoal cefoperazone deoxycholate agar (mCCDA) plates and incubated under microaerophilic conditions at 42 °C for 48 h (OIE, 2017) [11]. The presumptive colonies on mCCDA plates were subjected to genus identification by Gram stain and biochemical tests such as oxidase, catalase, and hippurate hydrolysis activity (Tenover and Fennell, 1992) [19].

Molecular confirmation by mPCR assay

The genomic DNA of *Campylobacter* suspected colonies were extracted by snap chill method (Swetha *et al.*, 2015) [18] and multiplex polymerase chain reaction (mPCR) was performed on nexus gradient mastercycler (M/s. Eppendorf, Germany) for molecular confirmation of *Campylobacter* species by targeting the lipid gene *lpx*A (Primers: *C. jejuni* (forward): ACAACTTGGTGACGATGTTGTA, *C. coli* (forward): AGACAAATAAGAGAGAATCAG, Reverse: CAATCATGDGCDATATGASAATAHGCCAT) (Klena *et al.*, 2004) [8] with an annealing temperature of 50℃. The mPCR yielded a product with a discriminatory amplicon size of 331 bp (*C. jejuni*) and 391bp (*C. coli*) on agarose gel electrophoresis (Amresco, USA). The mPCR products were stained using ethidium bromide and visualized under ultraviolet trans-illuminator and imaged using the Gel Doc IT system (UVP, UK).

Results and Discussion

Among fifty-four samples processed, three *C. coli* were isolated (5.55%). These isolates were characterised as circular, flat to slightly raised, grey coloured and spreading nature on mCCDA plates (OIE, 2017) [11] (Fig 1). This charcoal based medium could be successfully used without blood from live animals. This was in accordance with Engberg *et al.* (2000); Oyarzabal *et al.* (2005) [6., 12], who reported that mCCDA as an efficient medium with improved isolation rates in poultry. On Gram staining, all three isolates were typically gram negative, spirally rod or sea-gull shaped (Fig 2) and the isolates were oxidase and catalase positive (Fig 3 and 4) and negative for hippurate hydrolysis. These results were in harmony with Ramees, (2016); Kumar, (2016) [13, 9], where they also observed similar characteristic properties of *Campylobacter* on Gram staining and biochemical activity. On molecular characterisation by targeting *lpx*A gene, all the isolates yielded an amplicon size of 391 bp which was specific for *C. coli* (Fig 5). The *C. jejuni* and *C. coli* are the most important emerging foodborne infectious agent around the globe, responsible for frequent causes of human campylobacteriosis (WHO, 2018) [20]. Poultry and poultry products remain the number one cause of foodborne illness worldwide. The consumption of undercooked poultry products and the cross-contamination of carcasses and other food products continues to be a major public health concern (Epps *et al.*, 2014) [7]. After processing fifty-four caecal samples from free-ranging chickens collected from villages in Bareilly district, Uttar Pradesh, three *C. coli* (5.55%) were isolated. This was in accordance with the findings of Anderson *et al.* (2012) [2], where they reported an isolation rate of 6% (Two *C. coli* isolates) from domestic backyard chicken flocks in the Canterbury region of New Zealand and also with Nguyen *et al.* (2016) [10], who observed four *C. coli* isolates from cloacal and feacal samples of backyard poultry in Kenya and 6.5% isolation rate of *C. coli* from backyard chickens in Grenada (Sharma *et al.*, 2016) [16].

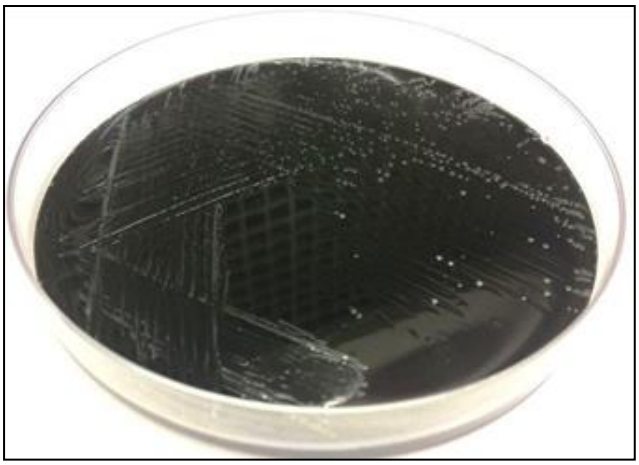

Fig 1: *Campylobacter* colonies on mCCDA agar

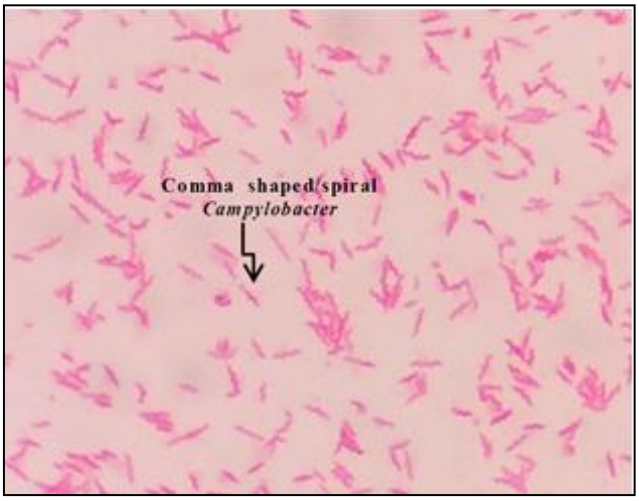

Fig 2: Gram stain

Fig 3: Oxidase test

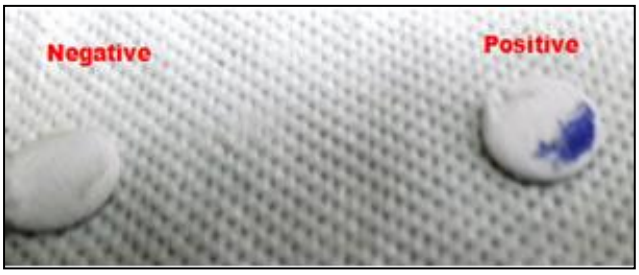

Fig 4: Catalase test

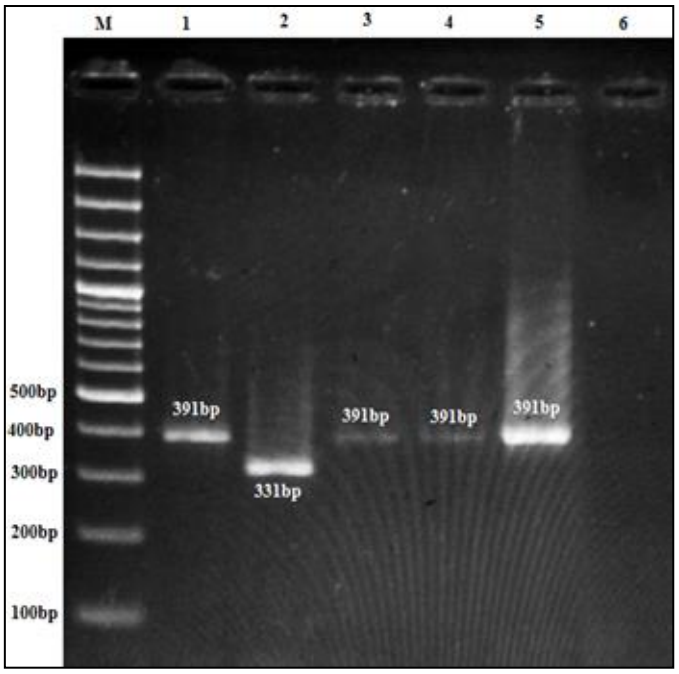

Fig 5: mPCR assay targeting *lpx*A gene

Lane M: 100 bp plus DNA ladder,
Lane 1: *C. coli* (391 bp) positive control,
Lane 2: *C. jejuni* (331 bp) positive control, Lane 3, 4 & 5: Field isolates positive for *C. coli* (391 bp), Lane 6: Negative control.

Conclusion

Campylobacter spp. is considered as a major cause of foodborne gastroenteritis around the world. In this study, *C. coli* were recovered from three chicken samples. The *C. coli* could result in severe foodborne infections and it leads to post infection sequeale like Guillain-Barré syndrome. Hence, it is highly recommended to educate the free-range chicken rearers about the application of good hygienic practices while rearing the chickens to prevent transmission of *Campylobacter* spp. from the environment to the birds. The elimination of *Campylobacter* infection requires a One Health approach and precise biosecurity measures. It is necessary to create awareness among the free-ranging chicken rearers about the principles of food safety and hygienic handling of birds while rearing, slaughtering and cooking to reduce the incidence of human campylobacteriosis.

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