OCCURRENCE OF *CAMPYLOBACTER* SPECIES IN CHICKEN EGG FROM CENTRAL KERALA

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ABSTRACT

Camylobacter is the leading cause of foodborne bacterial gastroenteritis worldwide. Campylobacter spp. are ubiquitous in the intestines of almost all poultry and wild birds. Poultry products are important source of human Campylobacteriosis. The main aim of this study was to screen the chicken eggs for the presence of Campylobacter spp. from retail outlets in central Kerala viz., Ernakulum, Palakkad and Thrissur. A total of 180 chicken egg samples, comprising 90 samples each were collected during two seasons, monsoon and summer. All the samples were subjected to isolation and identification by conventional culture technique. The charcoal based selective media modified charcoal cefoperazone deoxycholate agar was used for the isolation of Campylobacter spp. The occurrence of Campylobacter spp. in chicken egg samples was high in Ernakulum (13.3 per cent) district followed by Palakkad and Thrissur districts with 6.7 per cent each during monsoon season. In summer, highest occurrence was noticed in Palakkad district (6.7 per cent) followed by Ernakulum and Thrissur districts with 3.3 per cent each. The occurrence of Campylobacter spp. showed no significant difference between monsoon (8.9 per cent) and summer (4.44 per cent). An overall 6.7 per cent samples were positive for Campylobacter spp. in central Kerala. All the isolates were hippurate hydrolysis positive depicting C. jejuni as a main contaminant. Contamination of eggs with Campylobacter spp. is a threat to handlers and consumers, and is a major public health issue. The study necessitates the importance of Good Hygienic practices to be followed in the egg production chain to combat the Campylobacteriosis.

Keywords*: poultry, Campylobacter spp., hippurate hydrolysis.*

Foodborne diarrhoeal diseases are causing morbidity among 550 million people annually and 2,30,000 deaths. More than 33 per cent of these deaths are seen in children less than five years of age (WHO 2015). The incidence of human infections caused by *C. jejuni* and *C. coli* as the main bacterial agents of gastroenteritis has been increasing worldwide. The consumption of contaminated and undercooked foods mainly of animal origin are the source of human campylobacteriosis.

Poultry is one of the fastest growing sector in India. Eggs are a very good source of inexpensive, high quality protein containing all

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essential amino acids, fatty acids, vitamins A, D, E, choline, folate, calcium, magnesium, iron and zinc. India stands fifth in egg production and 17th in world poultry production (USDA 2014). Poultry is an important asymptomatic carrier of *Campylobacter* spp. Campylobacter infection mainly causes gastroenteritis in humans as well as in domestic animals. Septicaemia, bloody diarrhoea, vomiting, abdominal pain, fever and ulcerative colitis are characteristic symptoms in man. In about one in 1000 cases, the infection is followed by Guillain Barre Syndrome two to three weeks later, a debilitating inflammatory polyneuritis. Other possible autoimmune diseases from Campylobacter infections include Miller Fisher syndrome and Reiter's syndrome or reactive arthritis.

Poultry are major reservoirs of *Campylobacter* spp. but there is dearth of information regarding the occurrence of *Campylobacter* spp. in chicken eggs from retail outlets. By considering these factors the present study was undertaken with the objective of screening chicken eggs for the presence of *Campylobacter* spp. from retail outlets in central Kerala.

Materials and Methods

Collection of samples

A total of 180 chicken egg samples, comprising of 90 samples each were collected during two seasons, monsoon (June to September) and summer (February to May). In each season the egg samples were collected from three retail outlets each from three districts of central Kerala *viz*., Ernakulum, Palakkad and Thrissur. Eggs were collected aseptically and placed into individual air tight polythene covers sterilized by UV light. All the collected samples were immediately brought to the laboratory and were processed within four hours of collection to ensure that the organisms remain viable and culturable.

Processing of samples

Swabbing of intact eggs was done according to the procedure described by Evancho *et al.* (2001). A sterile cotton swab (HiMedia) was taken aseptically by grasping the end of the stick and moistened with phosphate buffered saline (HiMedia) and the excess diluent in the swab was removed by gentle pressing of the swab on the inside of test tube containing 30 ml sterile diluent. The swab handle was placed at 30° angle contact with the egg surface. The swab head was rubbed slowly and thoroughly over the surface three times, reversing direction between strokes. The swab head was then returned to the diluent tube and shaked vigorously, making 50 complete cycles in 10 sec, striking the palm of the other hand at the end of each cycle. This formed the triturate of the egg surface swab.

Isolation and identification of Campylobacter spp.

Twenty five millilitres of the egg surface swab triturate was enriched in 225 ml modified Charcoal Cefoperazone Deoxycholate (mCCD) broth (HiMedia). Loopful of the samples from mCCD broth were selectively plated on to Blood Free *Campylobacter* Selectivity (modified Charcoal Cefoperazone Deoxycholate, mCCD) agar (HiMedia) media supplemented with CAT (Cefoperazone, Amphotericin B and Teicoplanin) 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. The plates were incubated under microaerophilic conditions consisting of five per cent carbon dioxide at 42°C for 48h. Greyish, shiny, flat, moistened and mucoid colonies with tendency to spread, and with or without metallic sheen (Fig. 1) were selected for further

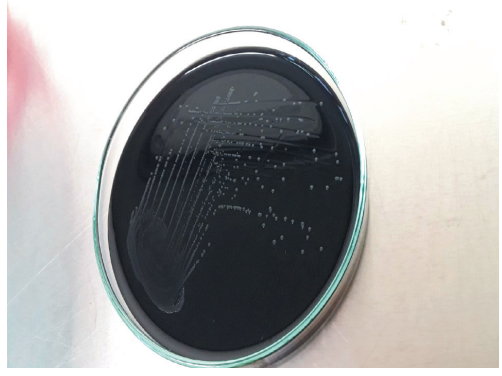

Fig. 1. *Campylobacter jejuni* on modified Charcoal Cefoperazone Deoxycholate agar plate

characterization. The suspected colonies of *Campylobacter* spp. were subjected to various biochemical test described by Tenover and Fennell (1992).

Statistical analysis

The data obtained were statistically analysed using the SPSS version 24.0. The significance of occurrence of *Campylobacter* spp. between different districts was analysed using chi-square with multiple proportion test. By using Z test for comparing two proportions, the significant difference during two seasons was analysed.

Results and Discussion

In a total of 90 chicken egg samples each analysed during monsoon and summer, 13.3 per cent samples from Ernakulum and 6.7 per cent samples each from Palakkad and Thrissur districts were positive for *Campylobacter* spp. in monsoon season (Table 2). In summer, 6.7 per cent samples from Palakkad district and 3.3 per cent samples each from Ernakulum and Thrissur districts yielded *Campylobacter* spp. (Table 3). In a total of 180 chicken egg samples analysed during two seasons, 8.9 per cent samples were positive for *Campylobacter* spp. in monsoon and 4.44 per cent samples in summer. Results obtained in the study are in accordance with the report of Renu *et al*. (2011) from Izatnagar, India and Joby (2016) from Thrissur, who observed highest occurrence of *Campylobacter* spp. during monsoon season with 10 and 6.67 per cent respectively when compared to summer. Renu *et al*. (2011) isolated four per cent and Joby (2016) could not isolate any *Campylobacter* spp. during summer. Rahimi and Tajbakhsh (2008) from Iran, Nather *et al*. (2009) from Germany and Vandeplas *et al*. (2009)) from Belgium reported higher rate in the occurrence of *Campylobacter* spp. during summer months with 66.5, 53 and 100 per cent occurrence rate respectively, which were contrary with the present study.

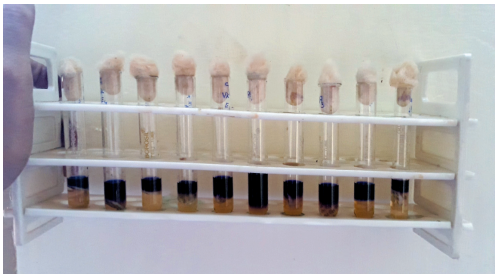

Fig. 2. Hippurate hydrolysis positive samples

The occurrence of *Campylobacter* spp. in chicken egg samples collected from three districts of central Kerala was statistically analysed and it was found that there was no significant difference between the occurrence of the organism from the three districts. By using Z test by comparing two proportions, the occurrence of *Campylobacter* spp. showed no significant difference between the two seasons, monsoon and summer.

Line (2006) reported that higher colonization of *C. jejuni* in chickens was promoted by high relative humidity in the environment, which might be a reason for higher occurrence for *Campylobacter* spp. during monsoon season. The average relative humidity of 85.56 per cent was noticed during monsoon season in central Kerala attributing to the increased occurrence of *Campylobacter* spp. in the present study.

The present study revealed that the overall occurrence of *Campylobacter* spp. in chicken eggs was 6.67 per cent which was in accordance with the results of Jonaidi-Jafari *et al*. (2016) who had isolated *Campylobacter* spp. from 7 per cent egg samples collected from supermarket in Iran. The reports of Messelhausser *et al*. (2011) from Germany and Joby (2016) showed 4.1 and 3.33 per cent occurrence of *Campylobacter* spp. in chicken eggs which were lower than the present study. Modirrousta *et al*. (2016) from Iran reported 31.6 per cent occurrence of *Campylobacter* spp. which was higher than the present study. Safaei *et al*. (2011) from Iran could not isolate any *Campylobacter* spp. from eggs collected from supermarket.

Higher occurrence of *Campylobacter* spp. in retail chicken eggs can be attributed to the contamination of the chicken eggs from excreta, dust and the litter material adhered to the egg shell surface. Contaminated plastic trays, environmental contamination

Table 1. Biochemical reactions of common *Campylobacter* spp.

Sl. No.	Tests	Biochemical reactions			
		C. jejuni	***C. coli***	***C. lari***	***C. upsaliensis***
1	Gram staining	-	-	-	-
2	Oxidase	+	+	+	+
3	Catalase	+	+	+	-
4	Motility	+	+	+	+
5	Aerobic growth test at 37°C	-	-	-	-
6	Growth at 25°C under microaerophilic conditions	-	-	-	-
7	Growth at 42°C under microaerophilic conditions	+	+	+	+
8	Hippurate hydrolysis	+	-	-	-
9	Indoxyl Acetate hydrolysis	+	+	-	+
10	H_2S on TSI agar medium	-	D	-	-
11	Sensitivity to Nalidixic acid	S	S	R	S
12	Resistance to Cephalothin	R	R	R	S

+ ➙ 90% or more of strains are positive; - ➙ 90% or more of strains are negative;
D ➙ 11-89% of strains are positive; R ➙ Resistant; S ➙ Susceptible.

Table 2. Occurrence of *Campylobacter* spp. in chicken egg in monsoon

Districts	Samples analysed	Positive samples	
		No.	Per cent
Ernakulum	30	4	13.30 [a]
Palakkad	30	2	6.70 [a]
Thrissur	30	2	6.70 [a]
Total	**90**	**8**	**8.90**

Figures bearing same superscripts do not differ significantly (p>0.05)

Table 3. Occurrence of *Campylobacter* spp. in chicken egg in summer

Districts	Samples analysed	Positive samples	
		No.	Per cent
Ernakulum	30	1	3.30 [a]
Palakkad	30	2	6.70 [a]
Thrissur	30	1	3.30 [a]
Total	**90**	**4**	**4.44**

Figures bearing same superscripts do not differ significantly (p>0.05)

and handling personal can act as sources of contamination.

All the isolates in the present study were subjected to biochemical tests (Table 1) and all the isolates showed hippurate hydrolysis (Fig. 2) indicating the contamination by *C. jejuni*. *Campylobacter jejuni* is the most common contaminant isolated from egg surface as *C. jejuni* colonisation in poultry is higher leading to faecal contamination of eggs (Parkar *et al*., 2013). In the present study 6.67 per cent chicken eggs collected from retail outlets of central Kerala were positive for *C. jejuni*. Joby (2016) from Thrissur reported that, all the isolates (3.3 per cent) obtained from retail eggs were *C. jejuni* positive. Thus the present study reveals that chicken eggs

from retail outlets can be a potential source of contamination by *C. jejuni*. Eggs reaching the retail markets should be properly washed in disinfectant solution at farm and should be stored under suitable conditions to prevent further contamination. An integrated approach of surveillance at both farm and retail outlets is necessary to reduce the contamination of eggs by *Campylobacter* spp. Effective surveillance of poultry, poultry products and humans is essential and forms part of the foundation of any consumer protection strategy.

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