Haryana Vet. (Dec., 2019) **58(2),** 245-247

Research Article

DETECTION OF *ESCHERICHIA COLI* FROM FOOD OF ANIMAL ORIGIN IN HISAR DISTRICT OF HARYANA

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Received: 27.06.2019; Accepted: 29.08.2019

ABSTRACT

The present study was undertaken to estimate the occurrence of *Escherichia coli* (*E. coli*) in various foods of animal origin from Hisar district in Haryana, India. A total of 130 samples comprising of bovine milk (raw -30 and pasteurized -20), ice-cream (30) and fresh chicken meat (50) were collected for the study. The results revealed that 74/130 (56.92%) of the samples were positive for *E. coli*. *E. coli* were isolated from 14/30 (46.67%) raw milk samples, 13/20 (65%) pasteurized milk samples, 6/30 (20%) ice-creams and 41/50 (82%) fresh chicken meat samples. On analysis, it was found that there was significant difference in isolation of *E. coli* from chicken meat and 'milk & milk products' with odds ratio - 6.49 (95% CI - 2.77-15.20; p <0.001). Isolation of *E. coli* from a large number of samples is worrisome as it indicates poor quality of products being sold in market, and calls for appropriate steps to be taken for improving the prevailing status of food production and processing.

Keywords: Chicken meat, *Escherichia coli*, Ice-cream, Pasteurized milk, Raw milk

Food of animal origin like milk and meat are the major part of human food, but their higher nutritive value makes them an ideal medium for rapid growth of bacteria especially under unhygienic production. Commonly, serious outbreaks of foodborne diseases have been traced back to consumption of animal originated food products contaminated by pathogens such as *E. coli*, Salmonella, *Listeria monocytogenes*, or *Campylobacter* spp. (Cerva *et al.*, 2014). Among food borne pathogens, *E. coli* is an opportunistic pathogen of humans and animals and is responsible for wide range of infections *viz*., diarrhoea, hemolytic uremic syndrome and hemorrhagic colitis (Bhoomika *et al.*, 2016). *E. coli* is used as an indicator of fecal contamination and indicates a possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard. The present study was carried out to detect *E. coli* in bovine milk and milk products and chicken meat.

MATERIALS AND METHOD

A total of 130 samples comprising of bovine milk (raw -30 and pasteurized -20), fresh chicken meat (50) and ice-cream (30) from the retail shops in and around Hisar city, Haryana were collected during September 2018 to February 2019. Samples were brought to laboratory immediately on ice and processed within 2-4 hours. All the collected samples were processed for isolation and identification of *E. coli* using standard methods as described earlier with certain modifications (Holt *et al.*, 1994). Briefly, collected food samples were inoculated in sterile MacConkey broth (1:10); mixed thoroughly and incubated at 37 °C for 24 h. A loopful of enriched inoculum was streaked on MacConkey lactose agar (MLA) and incubated at 37 °C for 24h. A loopful of small, round and pink colored colonies from MLA were streaked on Eosin methylene blue (EMB) agar and incubated at 37 °C for 24h. Dark-centered and flat colonies with metallic sheen were considered as *E. coli*. All the *E. coli* isolates were further confirmed by gram staining, biochemical tests and species specific Polymerase Chain Reaction (PCR) targeting phoA gene encoding Alkaline phosphatase (468bp) (Fig. 1) (phoA-F, GGTAACGTTTCTACCGC AGAGTTG, phoA-R, CAGGGTTGGTACACTGTCAT TACG) as described by Shome *et al.* (2011). PCR components included 12.5 µl Sapphire fast PCR- hot start master mix (2X), 0.5 µl of 10 µM of each primer, ~200 ng of template DNA and nuclease free water to make 25.0 µl. PCR was carried out using initial denaturation at 95 °C for 5 min, followed by 35 cycles of '94 °C for 30s, 57 °C for 45s and 72 °C for 45s' and final extension of 5 min at 72 °C. Electrophoresis of amplicons was performed in 1.5% (w/v) agarose gel, stained with ethidium bromide (0.5ìg/ml). The gel was analyzed under UV trans-illuminator and recorded using a Gel Documentation System (Alphaimager, HP).

All the statistical analyses were carried out using

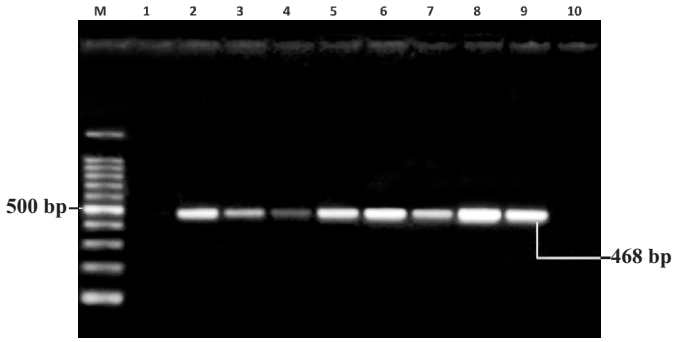

Fig. 1. Agarose gel showing PhoAgene amplified from *E. coli* isolates. Lane M-100bp DNA ladder, Lanes 1-9 - 468bp amplicons from *E. coli* isolates, Lane 10 - negative control

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Table 1

Isolation of *E. coli* from different samples of food of animal origin (n=130)

Type of sample			No. of samples	Positive	% positive	95% CI	odds ratio	95% CI	p-value
Chicken meat			50	41	82.00	68.56-91.42	6.49	2.77-15.20	<0.001
Bovine milk and milk products			80	33	41.25	30.35-52.81			
	Pasteurized milk		20	13	65.00	40.78-84.61	2.12	0.66-6.84	0.207
	Raw milk		30	14	46.67	28.34-65.67			
		Dairy	14	4	28.57	8.39-58.10			
		Vendors	16	10	62.50	35.43-84.80			
	Ice-cream		30	6	20.00	7.71-38.57			
Total			130	74	56.92	47.95-65.57			

STATA™ IC-15.0 (StataCorp, College Station, TX).

RESULT AND DISCUSSION

In the present study, out of 130 samples, 74 (56.92%) were found positive for the presence of *E. coli* (Table 1). The prevalence of *E. coli* was observed more in raw chicken meat samples than in 'milk & milk products' including pasteurized milk, raw milk and ice-cream. On analysis, a significant difference was observed in the prevalence of *E. coli* between meat, and 'milk & milk products' with odds ratio-6.49 (95% CI - 2.77-15.20; p<0.001), which indicates that there are 6.49 times chances of isolating *E. coli* from chicken meat as compared to milk & milk products. However, no significant difference was found between raw and pasteurized milk for the isolation of *E. coli* [odds ratio- 2.12; 95% CI=0.66-6.84, p=0.206] (Table 1).

For chicken meat, 82% (41/50) samples were contaminated with *E. coli*, which is comparable with the findings of previous studies in India. Hussain *et al.* (2017) reported 88.9% prevalence from different parts of India. However, varied results have been reported from other parts of the country. Zende *et al.* (2013) reported 16.67% contamination of *E. coli* in raw chicken meat from Mumbai, whereas Sharma and Chattopadhyay (2015) have reported a very high contamination of *E. coli* (98%) in raw meat samples from Kolkata. Similar results were reported by Odwar *et al.* (2014) from Kenya (78%). Many other studies worldwide have reported lesser carriage of *E. coli* in samples of poultry origin ranging from 1.53% in South Korea to 69.1% in China (Kim *et al.*, 2017; Wu *et al.*, 2014).

In the present study, 41.25% (33/80) milk and milk products were found to harbor *E. coli* including pasteurized milk as well as ice-cream. As regard to presence of *E. coli* in raw milk, 14/30 (46.67%) samples showed contamination with *E. coli*. There is wide variation in detection of *E. coli* from raw milk in India ranging from 8.57% in Mizoram (Karuppasamy *et al.*, 2015) to 81.1% in Chhattisgarh (Bhoomika *et al.*, 2016). Similar trend has also been observed in other countries, wherein Mashak (2018) reported 20% of raw milk samples from Iran to be contaminated with *E. coli* whereas Tabaran *et al.* (2017) reported 71.16% raw milk samples to be positive for *E. coli* in Romania.

The variation in isolation of *E. coli* from various regions may be due to variation in pre and post-production management practices being followed at the dairy farms, which predispose the milk to microbial contamination (Abunna *et al.*, 2019).

In the current study, 65% (13/20) of the pasteurized milk samples were found to be contaminated with *E. coli*. High prevalence of *E. coli* in pasteurized milk may be because of post pasteurization contamination or unhygienic measure at milk plant. Nazir (2011) has also reported similar results from Hisar, Haryana in which they found 80% of pasteurized milk samples to be positive for *E. coli*. Whereas, in another study in Goa, *E. coli* was detected from 26.4% of the pasteurized milk samples (Surve *et al.*, 2011). Contamination by *E. coli* was detected in 3.9% (El-Zubeir *et al.*, 2008) and 39.5% (Shojaei and Yadollahi, 2008) of pasteurized milk samples in South Africa and Iran, respectively.

In the present work, 20% (6/30) of branded ice-cream samples were found to have *E. coli*. Jadhav and Raut (2014) have reported similar results from Maharashtra in which they found 25% of ice-cream samples to be positive for *E. coli*. Meanwhile, lower detection rates were observed from Pantnagar (6.25%) (Kumar and Prasad, 2010). Studies from abroad have reported occurrence of *E. coli* in ice-cream to be as low as 0.06% in Libiya (El-Sharef

et al., 2006) to as high as 63.6% in Turkey (Caglayanlar *et al.*, 2009). The wide geographical variation in detection rates of *E. coli* in pasteurized milk andice-creamsmay be due to variationin the initial microbial load of raw milk/other ingredients, variation in the production techniques and post-production handling.

CONCLUSION

The isolation rate of *E. coli* was more from chicken meat samples as compared to 'bovine milk and milk products'. It highlights the differences in production and handling of these two types of food of animal origin. Further, isolation of *E. coli* from a large number of samples is worrisome as it indicates poor quality of products being sold in market and calls for appropriate steps to be taken for improving the production and handling techniques and post processing contamination.

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