The prevalence of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Campylobacter* spp. on bovine carcasses in Isfahan, Iran

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Summary

This study was carried out to determine the prevalence of foodborne pathogens, *Escherichia coli*, *E. coli* O157:H7, *Listeria monocytogenes* and *Campylobacter* spp. on slaughtered cattle in Isfahan, Iran. A total of 203 cattle carcasses were sampled by surface section of neck meat taken immediately after slaughter and analyzed using microbiological examinations. Suspected colonies to *E. coli* O157:H7 were confirmed by a specific polymerase chain reaction method (PCR). The results showed that the contamination rate of samples to *E. coli* and *E. coli* O157:H7 were 42.4 and 6.4%, respectively. Seasonal distribution showed that the highest prevalence of *E. coli* and *E. coli* O157:H7 occurred in summer samples. Six carcasses carried *L. monocytogenes* whereas *Campylobacter* spp. were not detected on any carcasses. The results indicated that prevalence of *E. coli* and *E. coli* O157:H7 was high on bovine carcasses in Isfahan. This condition should be considered as a probable hazard for human health.

Key words: Cattle, *Escherichia coli*, *E. coli* O157:H7, *Campylobacter*, *Listeria monocytogenes*

Introduction

The contamination of bovine carcasses during the slaughter and processing is a major risk for subsequent foodborne infections in humans. The most dominant pathogens involved i.e. *E. coli* O157, *Listeria monocytogenes*, and *Campylobacter* spp., are carried in the guts of cattle and shed in the faeces of the animals (Chapman *et al*., 1997; Wesley *et al*., 2000). The prevalence of these pathogens in cattle has been extensively studied in recent years. Typical prevalence rates between 0.12-27.8 (up to 68% in heifers), 0-15 and 5.0-53.0% have been reported for *E. coli* O157:H7, *Listeria monocytogenes* and *Campylobacter* spp. in cattle, respectively (Chapman *et al*., 1997; Tarr *et al*., 1999; Elder *et al*., 2000; Wesley *et al*., 2000; Chinen *et al*., 2001; Madden *et al*., 2001; Cagney *et al*., 2004). Prevalence rates of these pathogens, however, are subject to seasonal variation (Wesley *et al*., 2000).

Typical illness as a result of an *E. coli* O157:H7 infection can be life threatening, and susceptible individuals show a range of symptoms including haemorrhagic colitis and other complications, including haemolytic–uremic syndrome and thrombotic thrombocytopenic purpura (Bell *et al*., 1994).

Usually, a *Listeria* infection is linked to well defined high risk groups such as the immunocompromised individuals or pregnant women (Swaminathan, 2001). Individuals infected with *Listeria* may exhibit "flu-like" symptoms that can progress to septicemia, meningitis and meningoencephalitis in immunocompromised individuals, newborns and the elderly, and abortion and stillbirth in pregnant women (Farber and Peterkin, 1991). *Campylobacter* spp. are thought to be

the most common cause of bacterial gasteroenteritis worldwide (Humphrey *et al*., 1993).

The aim of this study was to evaluate the potential risk of carcass contamination of cattle in the slaughterhouse of Isfahan by determining the prevalence of the major foodborne pathogens, *E. coli* O157, *Listeria monocytogenes* and *Campylobacter* spp.

Materials and Methods

Carcass sampling

Samples were collected from three abattoirs in the Isfahan, Iran. Sampling was done monthly, over a period of 1-year (from April 2006 to May 2007). At each sampling visit, 4 to 7 cattle carcasses were sampled randomly, from each abattoir. A total of 203 carcasses were sampled after slaughter (post washing) and a section of neck meat, at least 10 × 10 × 0.3 cm, was aseptically removed using a sterile knife and placed in a sterile stomacher bag. The sample was held in a cool box during transport to the laboratory and analyzed within 24 h of excision.

Microbiological analysis

E. coli

Twenty five grams of each sample were homogenized in 225 ml tryptone soya broth (TSB, merck) supplemented with novobiocin (20 mg/l) and incubated at 37°C for 18-24 h. Following incubation, the enrichment samples were streaked onto levine eosin methylene blue (Levine-EMB) agar (merck) plates and incubated at 37°C for 18-24 h. Suspect colonies (green color with a metallic sheen) were streaked onto triple sugar iron (TSI, merck) for purity and screened by the indole test and triple sugar iron slants (Li *et al*., 2004; Stampi *et al*., 2004).

E. coli O157:H7

Twenty five grams of each sample were homogenized in 225 ml tryptone soya broth (TSB, merck) supplemented with novobiocin (20 mg/l). 100 µl of the concentrated sample was spread onto the surface of sorbitol MacConkey agar (SMA, merck) plates supplemented with cefixime (0.5 mg/l) and potassium tellurite (2.5 mg/l) and incubated for 18-24 h at 37°C. Suspect colonies (i.e. sorbitol negative) were streaked for purity and screened by indole test and then were confirmed as *E. coli* O157:H7 by polymerase chain reaction (PCR) assay using the O-antigen-encoding region of O157 gene and flagellar H7 gene (*fli C*) generic primers as described before (Gannon *et al*., 1997; Paton and Paton, 1998). Primer sequences used are shown in Table 1.

DNA was extracted from suspect colonies using a DNA extraction Kit (DNP™ Kit, Cinnagen Inc., Iran). Master circular gradient was used to amplify the fragment of O157 and H7 genes (Eppendorf Germany Co.). The reaction volume was 50 µl including 5 µl PCR buffer 10 x, 2 mM $MgCl_2$, 200 µM dNTP, 2 µM of each primer, 1 unit Taq DNA Polymerase (Roche Applied Science, Germany Co.) and 1 µg DNA of each sample. Thermal program was: 1 cycle of 94°C for 6 min, 34 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1 min and final extension of 72°C for 10 min (Sambrook and Russell, 2001).

Ten µl of PCR product was run in the gel electrophoresis to confirm the amplified fragment using agarose with ethidium bromide and size marker 100 bp (Fermentase), at constant voltage of 80.

Listeria monocytogenes

Before analysis, samples were stored at 4°C refrigerator. Twenty-five grams of each sample was mixed with 225 ml *Listeria* enrichment broth (LEB, merck), Homogenized, and then incubated at 30°C for up to 7 days.

Table 1: Primers used in PCR for detection of *E. coli* O157:H7

Primers	Direction	Primer sequence (5′ – 3′)	Product size (bp)	Reference
O157	Forward	CGGACATCCATGTGATATGG	259	Paton and Paton (1998)
	Reverse	TTGCCTATGTACAGCTAATCC		
fliC H7	Forward	GCGCTGTCGAGTTCTATCGAGC	625	Gannon *et al*. (1997)
	Reverse	CAACGGTGACTTTATCGCCATTCC		

On day 2 and 7, the enriched culture was streaked onto *Listeria* selective agar (LSA, merck) plates and incubated at 37°C for up to 48 h. The plates were examined for typical colonies of *Listeria* after 24 and 48 h incubation. Suspect colonies were identified by gram staining, growth on triple sugar iron (TSI, merck) agar, motility, catalase test, nitrate reduction test, haemolysis and CAMP test, as well as carbohydrate fermentation tests (rhamnose, xylose and manitol) (Doris and Seah, 1995).

Campylobacter spp.

Ten grams of each sample were added to 90 ml of Preston broth (PB, merck), supplemented with FBP (iron, bisulphite, pyruvate), then incubated (24 h, 42°C) in an anaerobic cabinet using a gas mix of 5% O_2, 10% CO_2, 85% N_2. Subcultures were then streaked onto *Campylobacter* selective agar (CSA, merck) and incubated as above. Plates were examined after 24 and 48 h and hanging drops prepared from characteristic *Campylobacter*–like colonies. When no typical colonies were observed, up to five other colonies were selected for microscopic examination. If typical spiral morphology and corkscrew-like motility were displayed, colonies were streaked onto blood agar and incubated as above to obtain pure cultures for speciation.

After initial characterization, presumptive *Campylobacter* spp. were confirmed using standard biochemical tests (Bolton and Robertson, 1982).

Appropriate positive and negative control cultures were included with all microbiological assays.

Statistical analysis

The isolation rates of foodborne pathogens at different seasons were compared by Pearson chi-square test. Differences in the isolation rate were considered statistically significant when $p<0.05$.

Results

The results of *Escherichia coli*, *E. coli* O157:H7, *Listeria monocytogenes* and *Campylobacter* spp. prevalence on beef carcasses are shown in Table 2.

Table 2: Occurrence of pathogenic bacteria on beef carcasses in Isfahan

Organism	Number of samples analyzed	Positive samples	
		n	%
Escherichia coli	203	86	42.4
E. coli O157:H7	203	13	6.4
Listeria monocytogenes	200	6	3.0
Campylobacter spp.	183	0	0.0

E. coli were detected in 86 (42.4%) of the 203 carcass samples and 13 samples (6.4%) were positive for both *E. coli* and *E. coli* O157:H7. Among 200 samples, only 6 samples (3.0%) were positive for *L. monocytogenes* and none of them were positive for *Campylobacter* spp. Only, one sample was positive for both *E. coli* and *L. monocytogenes*.

Statistical evaluation showed that there were significant differences ($P\leq0.05$) between the *E. coli*, *E. coli* O157:H7 and *Listeria monocytogenes* prevalence in different seasons (Table 3). The highest prevalence of *E. coli* and *E. coli* O157:H7 (60.3 and 14.8%, respectively) were in summer samples.

Listeria monocytogenes were only detected in spring and autumn samples with a prevalence of 4 and 8.2%, respectively (Table 3).

The extracted DNA from *E. coli* isolated from the samples were confirmed as *E. coli* O157:H7 by PCR assay using the O-

Table 3: The prevalence of *E. coli*, *E. coli* O157: H7 and *Listeria monocytogenes* on cattle carcasses in different seasons in Isfahan

Season	Number of isolates*		
	E. coli	*E. coli* O157:H7	*Listeria monocytogenes*
Spring	20/51 (39.2)^{a}	1/51 (2.0)^{a}	2/50 (4.0)^{a}
Summer	35/58 (60.3)^{b}	8/58 (14.8)^{b}	0/56 (0.0)^{a}
Autumn	22/49 (44.9)^{ab}	4/49 (8.2)^{c}	4/49 (8.2)^{b}
Winter	9/45 (20.0)^{c}	0/45 (0.0)^{a}	0/45 (0.0)^{a}

*Number of positive samples/number of samples examined (%). $^{a, b, c}$ Different letters show significant difference in the same column ($P\leq0.05$)

antigen-encoding region of O157 gene and flagellar H7 gene (*fli* C). PCR products are shown in Figs. 1 and 2.

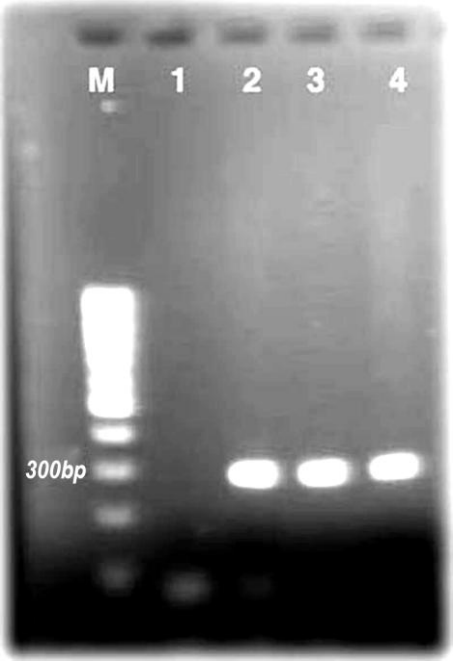

Fig. 1: PCR products of the samples for O157 gene (Column M = 100 bp DNA ladder (SM 0241, Fermentas Co.), Column 1 = negative control, Column 2 = positive control, Column 3, 4 = the positive samples)

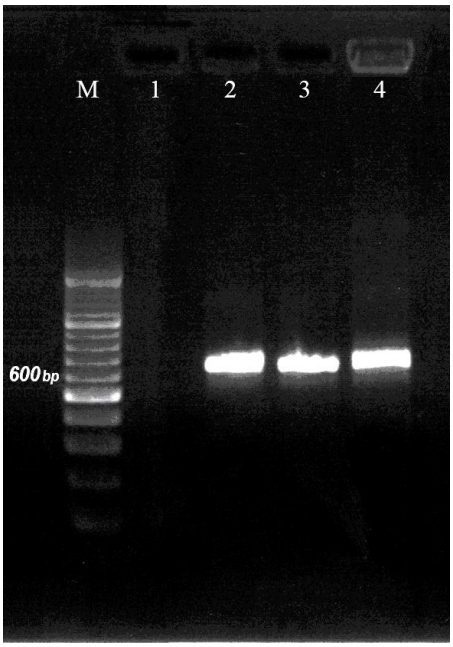

Fig. 2: PCR products for Flagellar H7 gene (Column M = 100 bp DNA ladder (SM 0321, Fermentas Co.), Column 1 = negative control, Column 2 = positive control, Column 3 and 4 = the positive samples

Discussion

Contaminated ground beef and other bovine products are particularly important in transmitting *E. coli* O157:H7. Since 1982, more than 100 outbreaks of enterohaemorrhagic *E. coli* O157 have been documented in USA (Elder *et al*., 2000). Fifty-two percent of these outbreaks have been attributed or linked to foods derived from cattle (Elder *et al*., 2000). Thus far, many studies have been completed to determine the prevalence of *E. coli* O157:H7/EHEC O157 on cattle carcasses (Heuvelink *et al*., 1998; Chapman *et al*., 2000; Madden *et al*., 2001).

In the current study, 6.4% of cattle carcasses were positive for *E. coli* O157:H7. The prevalence values reported in the present study in Iran was higher than Ireland (0.0%) (Madden *et al*., 2001), UK (1.1%) (Chapman *et al*., 2000), Italy (3.6%) (Conedera *et al*., 1997) and lower than Netherlands (10.4%) (Heuvelink *et al*., 1998), England (13.4%) (Chapman *et al*., 1997) and USA (28%) (Elder *et al*., 2000).

Many factors are contributed to the variations among the studies, including the improved enrichment and isolation procedures, differences in sample size, the type of sample and how it was collected, and when the samples were collected (i.e. seasonality) (Bryan *et al*., 2003). In this study the highest prevalence of *E. coli* O157:H7 was found on carcasses sampled in summer, which is in agreement with the previous studies that reported peak prevalence in the late summer and early fall (Hancock *et al*., 1997; Elder *et al*., 2000; Bryan *et al*., 2003).

In the present study, *L. monocytogenes* was detected on six cattle carcasses (3.0%). Studies conducted in Northern Ireland (Madden *et al*., 2001), UK (Fenlon *et al*., 1996), Australia (Vanderlinde *et al*., 1998) and the USA (Mc Namara, 1995) reported that 0.0, 7.0, 0.7-15.0 and 4.1%, of cattle carcasses were positive for *Listeria monocytogenes*, respectively. This study suggestsed that *Listeria monocytogenes* contamination of cattle carcasses in Isfahan, is low.

Campylobacter spp. were not detected from carcasses in this study (n = 183), but a

survey of beef carcasses (n = 120) in Tehran found that 10% were positive for *Campylobacter* (Taremi *et al*., 2005). In the USA, a survey of 2089 steer/heifer carcasses (Mc Namara, 1995) found that 4.0% carried *Campylobacter jejuni* or *Campylobacter coli*, but in Australia, Vanderlinde *et al*. (1998) found only 0.8% carcasses positive in domestic meat plants. Also, Madden *et al*. (2001) showed that the contamination rate of *Campylobacter* on beef carcasses in Nothern Ireland is less than 3%.

Overall, none of the 183 carcass samples yielded *Campylobacer* spp., and only 3/200 carcasses were positive for *Listeria monocytogenes*, therefore, the contamination rate is low for both, but the prevalence of *E. coli* and *E. coli* O157:H7 is higher than other studies.

Cattle are known to carry *E. coli* O157:H7 but the carcasses of carrier animals should be free of the organism unless abattoir practices permit cross–contamination from the hide or visceral contents to carcasses. Therefore, with good hygienic practices during skinning, dehiding and evisceration, the rate of carcasses contamination will be significantly low.

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