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PREVALENCE OF *LISTERIA* SPP., *CAMPYLOBACTER* SPP. AND *ESCHERICHIA COLI* O157:H7 ISOLATED FROM CAMEL CARCASSES DURING PROCESSING

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Summary

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The objective of this study was to determine the prevalence of the food-borne pathogens *Listeria*, *Campylobacter*, *Escherichia coli* O157:H7 isolated from camel carcasses during different stages of processing in a major commercial camel slaughterhouse in Iran. A total of 94 neck meat samples were taken from camel carcasses from 3 sites along the processing line including pre-evisceration, post-evisceration, and post-washing. The overall prevalence of *Listeria* spp., *Campylobacter* spp., and *E. coli* O157:H7 was 9.6%, 5.3%, and 1.1%, respectively. The prevalence of the organisms at pre-evisceration, post-evisceration and post-washing stages was 7.4%, 8.5% and 3.2% for *Listeria* spp., 1.1%, 2.1%, and 1.1% for *L. monocytogenes*, 3.2%, 4.3% and 1.1% for *L. innocua*, 3.2%, 5.3%, and 1.1% for *Campylobacter* spp., and 0.0%, 1.1%, and 1.1% for *E. coli* O157:H7, respectively. The prevalence of these organisms was different during different seasons. To our knowledge, this study is the first report on the prevalence of *Listeria spp.*, *C. jejuni, C. coli,* and *E. coli* O157:H7 isolated from camel carcasses in Iran.

Key words: camel carcasses, *Campylobacter* spp., *E. coli* O157:H7, food-borne pathogens, *Listeria* spp.

INTRODUCTION

Camel meat is nutritionally as good as that of the major sources of red or white meats and similar in taste and texture to beef. The amino acid and mineral contents of camel meat are of ten times higher than beef, probably due to lower intramuscular fat levels (Kadim *et al*., 2008). In addition, antibiotics and hormones are not used at sub-therapeutic doses or therapeutically in these animals compared to other food animals. This suggests that their microbiological flora may not be exposed to the same selective pressures as seen elsewhere in the meat industry.

There are three major pathogens that have frequently been associated with meat and meat products including *Campylobacter* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7. These organisms have been linked to a number of cases of human illness (Elder *et al*., 2000; Madden *et al*., 2001).

Campylobacter spp. are among the most common causes of acute enteric

diseases in humans throughout the world. The most important *Campylobacter* species associated with human illness are *C. jejuni* and *C. coli* (Wesley *et al.*, 2000). These organisms have frequently been associated with poultry, which are considered the primary source; however, other meats such as pork, lamb, and beef have also been implicated as sources of contamination (Taremi *et al*., 2006; Hussain *et al*., 2007).

Listeria monocytogenes is an important food-borne pathogen and has been associated with outbreaks and sporadic cases of listeriosis (Mead *et al*., 1999). Typical foodstuffs implicated as sources of the organism include salads and fermented meats and raw meats such as beef, pork, lamb, and poultry (Sheridan *et al*., 1994; Madden *et al*., 2001).

One of the most significant food-borne pathogens that has gained increased attention in recent years is *E. coli* O157:H7. 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 haemolytic colitis, hemolytic-uremic syndrome, and thrombotic thrombocytopaenic purpura. Typical sources of this pathogen have been identified and one of the primary hosts implicated are cattle (Bell *et al*., 1994; Chapman *et al*., 2000).

Overall, meat and meat products have been implicated as significant sources of all of the pathogens described above. The objective of this study was to determine the prevalence of the food-borne pathogens *Campylobacter* spp., *Listeria monocytogenes*, *Escherichia coli* O157:H7 isolated from camel carcasses during different stages of processing in a major commercial camel slaughterhouse in Iran.

MATERIALS AND METHODS

All media and chemicals were analytical grade purchased from Merck (Darmstadt, Germany) except those indicated.

Carcass sample collection and preparation

Two hundred eighty two samples were taken from the neck meat of 94 Iranian breed camel (*Camelus dromedarius*) carcasses at the Najaf-Abad slaughterhouse, Isfahan, Iran, from August 2007 to July 2008. This processing plant is the major animal plant in the Isfahan province which slaughters approximately 8–20 camels daily. Carcasses were randomly chosen and sampled on a weekly basis from three stages along the processing line including: pre-evisceration, post-evisceration and post-washing. A section of neck meat (10 cm × 10 cm × 3 cm) was aseptically removed and placed in a stomacher bag. The samples were immediately transported to the laboratory in a cooler with ice packs and processed within 24 hours.

Isolation and identification of Listeria

For isolation of *Listeria spp.,* samples were stored at 4 °C. A 25 g portion of each sample was mixed with 225 mL *Listeria* enrichment broth, homogenized and then incubated at 37 °C for up to 7 days. On the second and seventh day the enriched culture was streaked onto *Listeria* selective agar supplemented with Palcam *Listeria* selective supplement and incubated at 37 °C for up to 48 h. Then plates were examined for typical colonies of *Listeria*. Suspected colonies were identified using standard microbiological and biochemical procedure including Gram stain, growth on triple sugar iron (TSI) agar, motility, catalase, nitrate reduction, haemolysis, Christie, Atkins, Munch, Petersen (CAMP) test, as well as carbo-

hydrate fermentation tests for rhamnose, xylose and mannitol (Doris & Seah, 1995).

Isolation and identification of Campylobacter

Twenty five g of sample were added to 225 mL of *Campylobacter* enrichment broth (Himedia, Mumbai, India) supplemented with *Campylobacter* selective supplement (Himedia, Mumbai, India) and 25 mL defibrinated sheep blood for each 475 mL of media for pre-enrichment and incubated (42 °C, 24 h) in a microaerophilic environment (5% O_2, 10% CO_2, 85% N_2). Then sub-cultures were streaked onto *Campylobacter* selective agar (Himedia, Mumbai, India) supplemented with an antibiotic (polymyxin B, 2500 IU; rifampicin, 5.0 mg; trimethoprim lactate, 5.0 mg and amphotericin B, 5.0 mg) (Himedia, Mumbai, India) and 5% (v/v) defibrinated sheep blood and incubated for 48 h at 42 °C as described above. One presumptive *Campylobacter* colony from each selective agar plate was subcultured and tested by standard microbiological and biochemical procedure including Gram stain, catalase, oxidase tests and hippurate hydrolysis (Taremi *et al*., 2006).

Isolation of E. coli O157:H7

Twenty-five g of each sample were homogenized in 225 mL trypton soya broth supplemented with novobiocin (20 mg/L) and incubated at 37 °C for 18–24 h. Then the enrichment samples were streaked onto levine eosin methylene blue agar and sorbitol McConkey agar plates supplemented with cefexime (0.5 mg/L) and potassium tellurite (2.5 mg/L) and incubated as above. Suspected colonies were confirmed by TSI agar and indole, methyl red, Voges-Proskauer, citrate (IMViC) tests (Stampi *et al*., 2004). Sorbitol negative colonies were confirmed as *E. coli* O157: H7 with PCR assay by using the O-antigen encoding region of O157 gene (Paton & Paton, 1998) and flagellar H7 gene *(fli C)* generic primers as described previously (Gannon *et al*., 1997).

Statistical analysis

Data were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA). Using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), a Pearson chi-square test and Fisher's exact two-tailed test analysis was performed and differences were considered significant at values of $P<0.05$.

RESULTS

The prevalence of *Listeria* spp., *Campylobacter* spp., and *E. coli* O157:H7 isolated from camel carcasses during pre-evisceration, post-evisceration, and post-washing stages is summarized in Table 1. Of the 94 carcasses sampled, 9 (9.6%) were positive for *Listeria* spp. (carcasses which were positive at more than one sampling site were counted as being positive only once in overall prevalence). At the post-evisceration stage, the prevalence of the *Listeria* spp. was 8.5%. In pre-eviscerated and post-eviscerated samples it was more than four times greater than in post-washed samples (Table 1). The result showed that at the post-evisceration stage, 2 (2.1%) carcasses were positive for *L. monocytogenes*, 4 (4.3%) for *L. innocua*, 2 (2.1%) for *L. seeligeri*, and 1 (1.1%) for *L. ivanovii*.

At post-evisceration stage, 5 (5.3%) of 94 carcasses were positive for *Campylobacter* spp. (1 for *C. jejuni* and 4 for *C. coli*). In terms of overall prevalence, carcasses positive for *C. jejuni* and *C. coli were* 2 (2.1%) and 3 (3.2%), respectively.

Table 1. Prevalence of *Listeria* spp., *Campylobacter* spp., and *E. coli* O157:H7 isolated from camel carcasses during three stages of the processing line in a major slaughterhouse in Iran

Microorganism	Pre-evisceration	Post-evisceration	Post-washing
Listeria spp.	7.4% (7/94)	8.5% (8/94)	3.2% (3/94)
L. monocytogenes	1.1% (1/94)	2.1% (2/94)	1.1% (1/94)
L. innocua	3.2% (3/94)	4.3% (4/94)	1.1% (1/94)
L. seeligeri	2.1% (2/94)	2.1% (2/94)	0.0% (0/94)
L. ivanoii	0.0% (0/94)	1.1% (1/94)	0.0% (0/94)
Campylobacter spp.	3.2% (3/94)	5.3% (5/94)	1.1% (1/94)
C. jejuni	1.1% (1/94)	1.1% (1/94)	0.0% (0/94)
C. coli	2.1% (2/94)	4.3% (4/94)	1.1% (1/94)
E. coli O157:H7	0.0% (0/94)	1.1% (1/94)	1.1% (1/94)

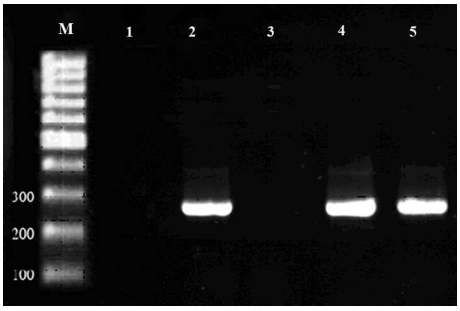

Fig. 1. PCR products of the samples for O157 gene. Column M: 100 bp DNA ladder, Fermentas Co.; column 1: negative control, column 2: positive control, column 3: negative sample, columns 4, 5: positive samples.

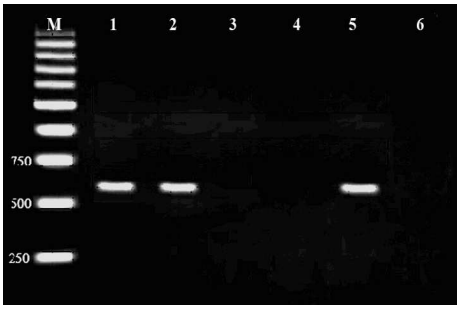

Fig. 2. PCR products of the samples for flagellar H7 gene. Column M=1 kb DNA ladder, Fermentas Co.; columns 1, 2: positive samples, columns 3, 4: negative samples, column 5: positive control, column 6: negative control.

The extracted DNA from *E. coli* positive samples was confirmed as *E. coli* O157:H7 by a PCR assay using the O-antigen-encoding region of O157 gene and flagellar H7 gene (*fli C*) (Fig. 1 and 2). Two *E. coli* O157:H7 were isolated: 1 (1.1%), at the post-evisceration and 1 (1.1%) at the post-washing collection sites.

Table 2 shows the prevalence of *Listeria, Campylobacter*, and *E. coli* O157:H7 on carcasses at different seasons. *E. coli* O157:H7 were only detected in summer with a prevalence of 2.9%. The highest prevalence for *Listeria* spp. and *Campylobacter* spp. were found in autumn (22.2%) and summer (11.8%), respectively.

DISCUSSION

The present study found that the prevalence of *Listeria* spp. and *L. monocytogenes* on post-washed camel carcasses was 3.2% and 1.1%, respectively. The presence of *L. monocytogenes* at the post-washing point is a food safety concern, as this pathogen is capable of growth at refrigeration temperatures (Hudson & Mott, 1993; Sheridan *et al*., 1994). It has

Table 2. Seasonal prevalence of *Listeria* spp., *L. monocytogenes, Campylobacter* spp., and *E. coli* O157:H7 isolated from camel carcasses in different seasons from a major slaughterhouse in Iran

Season	*Listeria* spp.	*L. monocytogenes*	*Campylobacter spp.*	*E. coli* O157:H7
Spring	10.5% (2/19)[a]	5.3% (1/19)[a]	5.3% (1/19)[a]	0.0% (0/19)[a]
Summer	2.9% (1/34)[b]	0.0% (0/34)[a]	11.8% (4/34)[b]	2.9% (1/34)[a]
Autumn	22.2% (4/18)[a]	5.6% (1/18)[a]	0.0% (0/18)[a]	0.0% (0/18)[a]
Winter	8.7% (2/23)[a]	0.0% (0/23)[a]	0.0% (0/23)[a]	0.0% (0/23)[a]

been indicated that food animals and meat products should be considered as a source for listeriosis in humans (Doris & Seah, 1995; Vanderlinde *et al*., 1998; Madden *et al*., 2001). To our knowledge, camel meat has never been reported as a source of listeriosis.

In this study, the prevalence of *Campylobacter* spp. was 5.3%, but only 1.1% of post-washed camel carcasses were positive. The presence of *Campylobacter* spp. in post-washed camel carcasses is still a food safety alarm as cross-contamination between carcasses may have occurred due to close proximity of the carcasses during chilling. In a study performed in Tehran, Iran by Taremi *et al*., (2005) 10% (12/120) of beef meat samples were positive for *Campylobacter* spp.; however, no *Campylobacter* spp. was isolated from 203 beef carcasses samples in Isfahan, Iran (Rahimi *et al*., 2008). In Australia, Vanderlinde *et al*., (1998) found only 0.8% carcasses positive in domestic meat plants.

Escherichia coli O157:H7 can cause severe disease and death in human beings (Elder *et al.,* 2000). Human infections of *E. coli* O157:H7 have mostly been recognized to be from food products of animal origin (Elder *et al*., 2000; Jo *et al*., 2004). Cattle have been implicated as the principal reservoir of *E. coli* O157:H7 (Chapman *et al*., 2000). Many studies determined the prevalence of *E. coli* O157:H7 on cattle carcasses which was from 0.0% to 27.8% (up to 68% in heifers) (Chapman *et al.*, 1997; 2000; Elder *et al*., 2000; Madden *et al*., 2001; Jo *et al.*, 2004). Many factors are thought to contribute to the variations among the studies, including the type of slaughtering, improved enrichment and isolation procedures, differences in sample size, the type of sample and how and when it was collected (Bryan *et al*., 2003). Therefore, we decided to determine the prevalence of *E. coli* O157: H7 in the camel carcasses. The results of this study showed that 1.1% (1/94) of camel carcasses was positive for *E. coli* O157:H7. This number is lower than that reported in cattle by Conedera *et al*. (1997) from Italy (3.6%), Rahimi *et al*. (2008) from Iran (6.4%), Chapman *et al*. (1997) from England (13.4%), and Elder *et al*. (2000) from the USA (28%). In this study *E. coli* O157:H7 was only detected on carcasses sampled in summer with a prevalence of 2.9%, which is in agreement with finding of previous studies on beef that reported peak prevalence occurs in summer and early fall (Elder *et al*., 2000; Bryan *et al*., 2003).

CONCLUSIONS

The current study is the first report on the prevalence of *Listeria spp.*, *C. jejuni/coli,* and *E. coli* O157:H7 on camel carcasses in Iran. Our findings provide some base-

line information regarding the prevalence of these important food-borne pathogens isolated from camel carcasses that could be used in future studies. The present study demonstrated that the prevalence of *Listeria*, *Campylobacter,* and *E. coli* O157:H7 were lower than that observed on beef. The study also suggests that camel meat may not be a significant source of the food-borne pathogens seen in other meat industries but monitoring programmes and inspection are necessary for preventing outbreaks of food-borne diseases.

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