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

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Summary


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
Prevalence of *Listeria* spp., *Campylobacter* spp. and *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-

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; Hussen 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, haemolytic-uremic syndrome, and thrombotic thrombocytopenic 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.
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% O₂, 10% CO₂, 85% N₂). 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 (flh 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.
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
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-
Prevalence of Listeria spp., Campylobacter spp. and Escherichia coli O157:H7 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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