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Prevalence of *Listeria* species in raw hamburger meatballs and chicken burgers in eastern Turkey

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The aim of this study was to determine the prevalence of *Listeria* spp. in raw hamburger meatballs and chicken burgers obtained from different fast-food and markets in Elazig and Malatya Provinces in the east of Turkey during 2010 and 2011. A total of 71 samples, consisting of 35 samples of raw hamburger meatballs and 36 samples of chicken burgers were investigated for the presence of *Listeria* spp. by culture method and a polymerase chain reaction assay targeting the listeriolysin O sequence for *L. monocytogenes*. We found that 34.3, 22.9, 5.7 and 5.7%, respectively of hamburger meatballs tested positive for *Listeria* spp., *L. innocua*, *L. monocytogenes* and *L. welshimeri*, respectively. However, 58.3, 33.3, 13.9, 8.3 and 2.8% of chicken burgers were found to be positive for *Listeria* spp., *L. innocua, L. monocytogenes, L. grayi* and *L. welshimeri,* respectively. *L. innocua* was isolated more frequently than the other species. This study shows the need for carefully evaluating the risk to acquire listeriosis from consumption of raw hamburger meatballs and chicken burgers.

Keywords: *Listeria* spp, *Listeria monocytogenes*, raw hamburger meatballs, chicken burgers, polymerase chain reaction.

INTRODUCTION

The hamburger is a special product consisting of minced meat together with additional ingredients (Tamminga et al., 1982). The U.S. Department of Agriculture's Food Safety and Inspection Service warns about the risk that raw hamburger may contain dangerous pathogenic bacteria, such as *Salmonella* spp., *Listeria monocytogenes* (*L. monocytogenes*), *Escherichia coli* (*E. coli*) O157:H7, *Staphylococcus aureus* (*S. aureus*) and *Campylobacter jejuni* (*C. jejuni*) (Richards, 2011).

Burgers are the world's most popular fast food, and they are made of either minced meat, minced chicken or fish paste with ingredients such as flour, oil, salt, pepper and some other preservatives (Wong et al., 2012). Consumption of burger has an increasing popularity, however, the safety of the burger patties is of great concern (Wong et al., 2012).

Invasive listeriosis is a rare, but often life-threatening, foodborne disease (Farber and Peterkin, 1991). According to the U.S. Centers for Disease Control (CDC), there are approximately 2500 human listeriosis cases per year and 500 of them may result in death (FDA-SFSAN, USDA-FIS, 2003). Listeriosis accounts for about 10% of all foodborne fatalities (Friedly et al., 2008). In ready-to-eat meat and poultry products, cross-contamination or re-contamination by *L. monocytogenes,* after a lethal thermal step, is of great concern, even when proper

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Abbreviations: HACCP, Hazard analysis and critical control points; **PCR,** polymerase chain reaction.

Table 1. Prevalence of *Listeria* spp. isolated from hamburger meatballs and chicken burgers.

Sample	Number of samples	*Listeria* spp. n (%)	*L. monocytogenes* n (%)	*L. innocua* n (%)	*L. grayi* n (%)	*L. welshimeri* n (%)
Hamburger meatballs	35	12 (34.3)	2 (5.7)	8 (22.9)	-	2 (5.7)
Chicken burgers	36	21 (58.3)	5 (13.9)	12 (33.3)	3 (8.3)	1 (2.8)
Total	71	33 (46.5)	7 (9.9)	20 (28.2)	3 (4.2)	3 (4.2)

hazard analysis and critical control points (HACCP) and thermal treatment procedures are followed (Friedly et al., 2008).

Although the presence of *Listeria* spp. in raw hamburger meatballs and chicken burgers is known, there is little information regarding this subject in Turkey. Therefore, the objective of the present study was to analyze the prevalence of *Listeria* spp. in raw hamburger meatballs and chicken burgers on sale in Elazig and Malatya Provinces in eastern Turkey.

MATERIALS AND METHODS

Samples

A total of 71 samples consisting of 35 samples of raw hamburger meatballs and 36 samples of chicken burgers from different fast-food and markets in Elazig and Malatya Provinces in the east of Turkey during 2010 and 2011 were examined for the presence of *Listeria* spp. The samples were taken under sterile procedures, kept in a container and immediately transported to the laboratory, where they were processed within 2 h.

Isolation and identification of *Listeria* species

Listeria isolation was performed according to method recommended by the United States Department of Agriculture (USDA) and Food Safety and Inspection Service (Anon, 1994; Dever et al., 1993). Twenty-five grams of samples were homogeneized into 225 ml of Listeria Pre-Enrichment Broth (Oxoid) using a stomacher for 2 min. Following incubation for 48 h at 30°C, 1 ml of this mixture was transferred into tubes containing 9 ml of University of Vermont Medium-Modified Listeria Enrichment Broth (UVM 1). The tubes were then incubated for 24 h at 35°C. At the end of this incubation period, a 1 ml of the UVM 1 medium was transferred into UVM 2 medium. After incubation at 35°C for additional 24 h, an aliquot of 0.1 ml of the UVM 2 medium was inoculated directly onto Listeria Selective Agar (Oxoid) and incubated for 48 h at 35°C. The suspected colonies were subcultured in Tryptic Soy Agar-Yeast Extract (Difco) medium for pure culture. The suspected colonies were then assayed by Gram staining, typical umbrella motility, catalase test, mannitol, rhamnose and xylose fermentation, nitrate reduction, β-haemolytic activity, and Christine-Atkins-Munch-Petersen (CAMP), according to Bergey's Manual of Systematic Bacteriology (Seeliger and Jones, 1986; Erol and Sireli, 1999; Yucel et al., 2005; Gebretsadik et al., 2011).

L. monocytogenes DNA preparation and PCR

L. monocytogenes genomic DNA was prepared using the QIAamp DNA mini kit (Qiagen, Germany) according to the recommendations of the manufacturer. The extracted *L. monocytogenes* DNA was kept at -20°C for PCR.

A final volume of 50 µl of the PCR mixture contained 5 µl of 10 X PCR buffer (750 mM Tris-HCl, pH 8.8, 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20), 5 µl of 25 mM magnesium chloride, 250 mM each of deoxyribonucleotide triphosphate, 1.25 U of Taq DNA polymerase (MBI Fermentas, St Leon-Rot, Germany), 20 pmol of each primer [LM1 (5'-CCTAAGACGCCAATCGAA -3') and LM2 primers (5'-AAGCGCTTGCAACTGCTC -3')] targeting the listeriolysin O sequence (Border et al., 1990), and 5 µl genomic DNA. The reaction mixture was subjected to the following cycling conditions in a touchdown thermalcycler (Hybaid, England): first denaturation at 94°C for 4 min, denaturation at 94°C for 30 s, annealing at 52°C for 1 min and extension at 72°C for 1 min 30 s. After 30 cycles, a final cycle comprising a 7 min extension step at 72°C (Border et al., 1990). Ten microliter amounts of amplified products were loaded onto 1.5% (w/v) agarose gels, subjected to electrophoresis, stained with ethidium bromide (0.5 µg/ml) and examined under an ultraviolet light. The DNAs of some isolates of *L. monocytogenes* obtained from our previous study (Ozbey et al., 2006) and distilled water were used as positive controls and negative control in all PCR tests, respectively.

RESULTS AND DISCUSSION

A detailed distribution of samples testing positive for *Listeria* spp. is shown in Table 1. Of 35 hamburger meatballs analysed, 8 (22.9%), 2 (5.7%) and 2 (5.7%) isolates were identified as positive for *L. innocua, L. monocytogenes* and *L. welshimeri*, respectively. Among the 36 chicken burgers tested, 12 (33.3%), 5 (13.9%), 3 (8.3%), and 1 (2.8%) isolates were found to be positive for *L. innocua, L. monocytogenes, L. grayi* and *L. welshimeri*, respectively. All isolates identified as *L. monocytogenes* yielded by PCR the specific fragment of 701 bp.

Recently, there is a growing interest regarding the huge increase in the consumption of "fast food". Previous studies carried out in Turkey have reported the presence of some pathogens in hamburgers collected from different retail markets (Agaoglu et al., 2000; Keles et al., 2006; Icoz and Kayisoglu, 2012). Although a high prevalence of *L. monocytogenes* in many food products is reported worldwide, the epidemiological data on

the prevalence of pathogenic *L. monocytogenes* in raw hamburger meatballs and chicken burgers in Turkey are insufficient. Thus, this study aimed to determine the prevalence of *Listeria* spp. in raw hamburger meatballs and chicken burgers from different fast-food and markets. Previous studies carried out in several countries have reported high microbial counts and the presence of pathogens in frozen hamburgers (Coelho and Barbosa, 1993; Gill et al., 1997; Tamminga et al., 1982; Torner et al., 1995). Research findings reported by Coelho and Barbosa (1993) and Gill et al. (1997) showed that hamburgers produced at the retail level may have lower microbiological quality. Undercooked hamburgers were implicated in foodborne disease outbreaks caused by *Salmonella* spp. and *E. coli* O157:H7 (Fontaine et al., 1978; CDC, 1994, 1997). Moreover, the ability of the microorganism to survive to frozen storage and multiply in freezing conditions (Gianfranceschi and Aureli, 1996; Palumbo and Williams, 1991) may account for the presence of *L. monocytogenes* in frozen ground beef and hamburgers (Farber and Peterkin, 1991; Sheridan et al., 1994).

Wong et al. (2012) investigated three different types of burger patties and found that the prevalence of *L. monocytogenes* in chicken patties (33.3%) was higher than that of beef (22.9%). In a study carried out in Turkey, 40, 26.6, 23.3, 10 and 3.3% from 30 chicken burgers were identified as *Listeria* spp., *L. monocytogenes, L. innocua, L. grayi* and *L. Welshimeri*, respectively (Sireli et al., 2002). *Listeria* spp. were found in higher rate (58.3%) in this study, unlike a previous study by Sireli et al. (2002). This may be due to poor hygiene and sanitation during production, process, storage and transport of hamburger meatballs and chicken burgers. In this study, *L. innocua* was isolated from hamburger meatballs with a higher prevalence (33.3%) than *L. monocytogenes*. Also, *L. innocua* was found to be more prevalent than other species (*L. monocytogenes, L. welshimeri* and *L. grayi*) in chicken burgers. *L. innocua* is no pathogenic for humans, and this suggests that *L. innocua* might be a very common organism in the environment (El-Shenawy and El-Shenawy, 2006). Seeliger (1988) demonstrated that *L. innocua* is a good indicator for *L. monocytogenes*. Thus, when looking for sources of *Listeria* the presence of both of these species could be managed as equally significant (Seeliger, 1988).

Published studies used specific PCR primer sets targeting genes such as the listeriolysin O gene (Border et al., 1990; Thomas et al., 1991), the Dth18 gene (Wernars et al., 1991), and the invasive associated protein (*iap*) gene (Bubert et al., 1992). We used specific primers for the listeriolysin O gene. All *L. monocytogenes* strains which were positive by the biochemical identification tests generated specific amplicons of 701 base pairs by PCR.

In conclusion, we describe the presence of *Listeria* spp. in hamburger meatballs and chicken burgers and suggest there is a potential risk for public health from consumption of raw or under-cooked hamburger meatballs and chicken burgers. The entity of this potential risk as well as the most effectives strategies to control *Listeria* in hamburger meatballs and chicken burger products have to be investigated in future studies.

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