The microbiological quality of ground beef in Aydin and Afyon Provinces, Turkey

B. SIRIKEN

Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Afyon Kocatepe, Afyon, TURKEY e-mail: bsiriken@yahoo.com

SUMMARY

To determine the microbiological quality of ground beef, a total of 70 ground beef samples were randomly purchased from various butchers and markets in Aydin and Afyon provinces, in Turkey between 2001-2002. According to analysis, 79% of the samples contained >10^3 aerobe mesophile plate count, 44% >10^2 cfu/g Pseudomonas, 47% >10^3 cfu/g enterobacteriaceae, 65% >10^3 cfu/g enterococci, 42% >10^3 cfu/g micrococi/staphylococci and 64% contained at >1100 MPN/g coliforms. 21.4% of the samples were positive with regard to coagulase positive staphylococci and, among them 5.7% were above 10^5 cfu/g. E. coli was detected in 30% of samples and, 20% of them were above 9.44 MPN/g. Salmonella spp. were detected in 10% of the samples and, the numbers in the positives were in 0.3-1100 MPN/g. The pH values measured were varied from 4.48 to 6.05. The results indicate that, the microbiological quality of the ground beef samples analyzed was unsatisfactory, and the product could be an important cause of food poisoning. Preventative measures include warning consumers of the health risks associated with eating raw ground beef and encouraging them to thoroughly cook ground beef and to adhere to safe food handling guidelines. In addition, good manufacturing practices (GMP) for slaughtering and processing of ground beef should be accepted as strategies to control pathogenic bacteria.

Keywords: Ground beef - microbiology - quality.

RéSUMÉ

Qualité microbiologique du bœuf haché dans les provinces turques d’Aydin et d’Afyon en Turquie. Par B. SIRIKEN.

Afin de déterminer la qualité microbiologique du bœuf haché, un total de 70 échantillons de bœuf haché ont été achetés aléatoirement chez divers bouchers et sur des marchés des provinces d’Aydin et d’Afyon, en Turquie entre 2001-2002. Selon des résultats d’analyses, 79% des échantillons contenaient plus 10^3 bactéries mesophiles aérobies par gramme, 44% > 10^3 cfu/g Pseudomonas, 47% > 10^3 cfu/g entérobactéries, 65% > 10^3 cfu/g enterococques, 42% > 10^3 cfu/g micrococi/staphylococci et 64% des échantillons contenaient plus de 1100 MPN/g de coliformes. 21.4% des échantillons étaient positifs en ce qui concerne les staphylocoques a coagulase positive et, parmi eux 5.7% étaient au-dessus de 10^5 cfu/g. E. coli a été détecté dans 30% d’échantillons et, 20% d’entre eux étaient au-dessus de 9.44 MPN/g. Les Salmonelles spp. ont été détectées dans 10% des échantillons et les nombres dans les échantillons positifs étaient de 0.3-1100 MPN/g. Les valeurs du pH mesurées variaient de 4.48 à 6.05. Ces résultats indiquent que, la qualité microbiologique des échantillons de bœuf haché analysés était insuffisante, et que ce produit pourrait être une cause importante d’intoxication alimentaire. Les mesures préventives incluent d’avertir les consommateurs sur les risques sanitaires liés à la consommation du bœuf haché cru, d’encourager à faire bien cuire la viande de boeuf hachée et de suivre les guides de bonnes pratiques concernant la manipulation des aliments. De plus, pour réduire la contamination par des microbes pathogènes, il faut mettre en place les mesures de bonnes pratiques au stade de la fabrication (GMP).

Mots-clés : Bœuf haché - microbiologie - qualité.

Introduction

Animal products, including carcasses and fresh meat, are easily contaminated with microorganisms and support their growth if not properly handled, processed and preserved [35,40]. A variety of sources, including air, water, soil, feces, feed, hides, intestines, lymph nodes, processing equipment, utensils and humans, contribute to the microbial contamination of the sterile muscles of healthy animals during slaughter, fabrication, and further processing and handling [6,28].

Raw ground meat is also a good medium for the rapid growth of microorganisms. The bacteria normally found on the surface of meat are distributed throughout the entire product during the mincing and mixing process used to produce raw ground meat [48]. It has been known as a vehicle for transmission of organisms such as E. coli and S. aureus [6,15] and, undercooked ground beef is also the most frequently recognized vehicle for Escherichia coli O 157:H7 infection [13]. Salmonella spp. infections traced back to beef products, particularly ground beef [30,33] have increased the public’s concern for the safety of all beef products.

Contamination of beef trimmings increases as the product progresses through the grinding and comminuting process. This is due to several factors, including increased product temperature, product homogenization and greater exposure to contamination. The final bacterial population of these products is, however, related to the initial contamination on the raw materials and single sources of heavy contamination can cross-contaminate numerous final product batches of ground beef [31,38]. Inadequate cleaning and sanitization of the meat grinder also may result in ongoing contamination of ground beef over many production days. Contamination with spoilage microorganisms may lead to product and economic losses, while presence of pathogens or their toxins may be the cause of foodborne disease that may lead to loss of human life [35].

Ground beef a raw food of animal origin forms a significant portion of the diet of the Turkish people. Consumers expect meat products to be safe for consumption when handled and cooked properly. However, like any other raw food, ground beef may be contaminated during production, processing, storage and marketing with biological agents that may be hazardous to human health. Studies worldwide and in other parts of Turkey have shown that Salmonella, E. coli, S. aureus, Listeria spp., Campylobacter and other pathological bacteria are often present in ground beef. However, there is less data concerning the prevalence of contamination with pathogen and other hygienic indicator microorganism of...
Material and Methods

A total of 70 ground beef samples were randomly purchased from various butchers and markets between 2001-2002, in Aydin (40 samples), and Afyon (30 samples), provinces. Two different methods were used for the determination of bacteria. The total aerobe mesophile plate count (APC), micrococci/staphylococci, coagulase positive staphylococci, enterobacteriaceae, enterococci and Pseudomonas counts were obtained by drop plating technique and reported as colony forming units (cfu) per gram. Total coliform counts (TCC), E. coli counts (ECC) and Salmonella spp. were determined using Most Probable Number (MPN) technique estimate of bacterial population density per gram.

For this purpose, a 10 g ground beef sample was transferred to a sterile plastic bag under aseptic conditions. The sample was then diluted to a $10^{-1}$ dilution with Peptone Water (Oxoid CM 9, UK) and stomached for 2 min by using a Stomacher (Interscience-Bag Mixer 400). Following homogenization, ten-fold serial dilutions for each samples were made in steril peptone-salt water up to $10^{-7}$. Each of these dilutions was inoculated to specific culture media for isolation of APC (Plate Count Agar, Oxoid CM 325, UK) [3], micrococcci/staphylococci, coagulase positive staphylococci (Baird Parker Medium, Oxoid CM 275, UK) [4], enterobacteriaceae (Violet Red Bile Glucose Agar, Oxoid CM 485, UK), enterococci (Slanetz and Bartley Medium, Oxoid CM 377, UK) [4], Pseudomonas (Pseudomonase Agar Base, Oxoid CM 559, CFC Selective Suppl. Oxoid SR 103, UK).

Colonies on plates were manually counted and reported in base ten logarithms of colony forming units per gram of sample (Log 10 cfu/g). These microorganisms were identified after isolation by Gram staining and appropriate biochemical tests (oxidase test-Oxoid BR 64 for Pseudomonas identification, and coagulate test for coagulate positive staphylococci identification).

For the isolation of coagulate positive staphylococci, up to 5 typical colonies (black or grey colonies) grown on BP Agar were selected and, transferred to tubes contained Brain Heart Infusion Broth (BHI-Oxoid CM 225, UK). The tubes were incubated at 37°C for 24 h. After the incubation, coagulate tests were done [44].

For the isolation of Salmonella spp., 4 fermentation tubes of 10, 1, 0.1, 0.01 g samples were added to Buffered Peptone Water (BPW) for a pre-enrichment step. The tubes were incubated at 37°C for 24 h [3]. Then, 0.1 ml from each BPW was added to Rappaport Vassiliadis (RV) Enrichment Broth (Oxoid CM 669, UK) for selective enrichment [47]. The tubes were incubated at 43°C for 24 h. After incubation, one loopful of each enrichment broth was streaked onto Brilliant Green (Modified) Agar (Oxoid CM 329, Suppl. SR 87, UK). After incubation, a presumptive colony of Salmonella was chosen and identified using Gram staining, appropriate biochemical tests (Triple Sugar Iron Agar-Oxoid CM277, UK, Lysine Iron Agar-Oxoid CM381, UK, urease test -Oxoid CM53, UK and, Simmons Citrate-Oxoid CM155, UK) and then tested using Salmonella polyvalent antiserum (Difco 2264-47-2).

For the isolation of TCC, 3 fermentation tubes were set up each containing 1, 0.1, 0.01 g sample in 10 ml Lactose Broth (LB-Oxoid CM 137) for a pre-enrichment step. The tubes were incubated at 37°C for 24-48 h. After incubation, a loop-full of inoculum from each tube with turbidity and gas production was transferred to Brilliant Green 2 % Bile Broth (BGBP-Oxoid CM31 UK) and incubated at 37°C for 24-48 h for a selective enrichment step. After incubation, BGBP tubes with turbidity and gas production were evaluated TCC. To identify E. coli, each positive BGBP tube was streaked onto Endo Agar Base (Oxoid CM 479, UK). After incubation, colonies on the Endo Agar base were evaluated for E. coli and finally, presumptive E. coli colonies were chosen and subjected to IMViC (Indol, Metil red, Voges Proskauer and Simmon Citrate tests).

Results

The results of the microbiological analysis of the ground beef samples are shown in Tables I and II. The number of APC and Pseudomonas (Table I) were found to be $>10^5$ and $>10^6$ cfu/g in 79 % and in 44 % of the samples, respectively. 47.2 % of the samples contained $>10^3$ cfu/g enterobacteriaceae, 65.7 % $>10^3$ cfu/g enterococci and, 42.8 % $>10^3$ cfu/g micrococci/staphylococci (Table I). Coagulase positive staphylococci (found in 21.4 % of the samples) were $>10^3$ cfu/g in 5.7 % of the samples (Table II). Coliforms bacteria were present in all the samples and, 64.3 % of them contained $>1100$ MPN/g (Table I). E. coli was detected in 30 % of samples and 20 % of them were above 9.44 MPN/g. The contamination level of Salmonella spp. was 0.3-1100 cfu/g in 10 % of the samples (Table II). The pH values varied from 4.48 to 6.05.

Discussion

The present study demonstrated that three pathogenic microorganisms were present in retail ground beef obtained from butchers and supermarkets in Aydın and Afyon provinces. Additionally, ground beef was also contaminated with other enteric and spoilage bacteria.

APC of 4.1-8.4 log_{10} organisms per gram of ground beef have been reported by various workers in different geographical areas [22,24,25,51]. In this study, it was observed that the APC in ground beef were similar to the findings reported by earlier investigations in other geographical regions [19,29,51]. For instance, more than 79 % of the samples contained $>10^5$ APC. Similarly, Heredia et al (19) reported that over 75 % of 88 samples contained $>10^5$ total mesophilic microorganisms per g. In this study, it was also found that 44 % of samples contained $>10^3$ Pseudomonas, and about 46 % contained $>10^4$ enterobacteriaceae. Mousa et al (24) reported that the mean values of total mesophilic and enterobacteriaceae counts were 7.2 x 10^8 and 4.3 x 10^7, respectively. Uzunlu (46) reported that Pseudomonas spp. was isolated 4.6 x 10^4 kob/g from ground beef. The presence of these
bacteria indicates that general hygiene and especially the high level of APC and *Pseudomonas* counts affect the shelf-life of ground beef. Spoilage bacteria such as *Pseudomonas* are generally not harmful but they will cause food to deteriorate or lose quality by getting mouldy, developing a bad odor, or feeling sticky on the outside [34]. In addition, several investigators have reported that correlation coefficients of aerobic plate, total coliforms counts, and *E. coli* counts with *Salmonella* incidence were higher [34, 37].

Another finding of this study, is that 65 % of ground beef samples contained >10⁴ cfu/g enterococci. Youssef et al (48) reported that the enterococcal population ranged from 10² to 5.1 x 10⁴ cfu/g with a mean number of 7.7 x 10³ cfu/g. Contamination of ground beef with *Salmonella* is still considered a major problem in food hygiene. Several studies have indicated that *Salmonella* spp. are present in beef carcasses, and the reported rates of contamination vary from 0.2 to 21.5, with a median of 3.3 % [9,29,39]. The prevalence of *Salmonella* spp. in ground beef is considerably higher and ranges from 0 to 45.2 %, with a median of 9.7 % [1,10,11,16,19,20,24,26,43,48,51]. In these studies, the researchers reported that *S. Typhimurium* [41,51] and *S. Typhi* [12] were the most identified serotypes. Ionova et al (20) reported that 1.81 % of 14.188 minced meat samples were contaminated with as many as 17 serotypes of *Salmonella* organisms belonging to the B, C and E serogroups. Fukushima et al (16) also reported that 125 ground beef samples were examined for the presence of *Salmonella* spp. and Sal biogroup 1 was found in 8.3 % and Sal biogroup 2 in 0.8 % of beef. *Salmonella* was counted at ≤10²/100 g or less. Similarly, the results of this study indicate that *Salmonella* spp. contamination in ground beef was detected in 10 % (7 samples), and contaminated with *Salmonella* spp. of the level of 0.3-1100 MPN/g. There was a wide variation in *Salmonella* spp. in ground beef throughout the different areas in the world as reflected by the above-mentioned results. The differences could be due to seasonal factors, geographic area, analyzed sample number and isolation method. Many researchers reported that the season was a factor in contamination of ground beef with *Salmonella* spp. the contamination rate increasing with the outside temperature [21, 32].

Several studies have indicated that coliforms and *E. coli* are present in ground beef [15,19,20,24,51]. Coliform bacteria are indicator organisms as enterobacteriaceae are of intestinal origin. Indicator organisms may be employed to reflect the microbiological quality of foods relative to product shelf life or their safety from foodborne pathogens. Microbial indicators are more often employed to assess food safety and sanitation than quality [7, 45]. Microbial contamination of ground beef with coliforms in this study was present in all of the samples and 64.3 % of the samples contained >1100 MPN/g whereas *E. coli* was detected in 30 % of samples and, 20 % of samples contained >9.44 MPN/g. Another study in Turkey by Uzunlu (46) reported that *E. coli* was isolated <3.0 x 10² from ground beef. In this study, the isolated *E. coli* percentage was very high. Although all isolates belonged to biotype 1, none of *E. coli* isolates identified in this study was positive or negative for virulence-associated Shiga toxin or enterotoxin genes. There are a few reports

<table>
<thead>
<tr>
<th>Log₁₀ cfu/g</th>
<th>APC (%)</th>
<th>CFC (%)</th>
<th>VG (%)</th>
<th>SB (%)</th>
<th>BP (%)</th>
<th>CPS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2.0 x 10⁰</td>
<td>0 (0.0)</td>
<td>23 (32.85)</td>
<td>4 (5.71)</td>
<td>0 (0.0)</td>
<td>3 (4.28)</td>
<td>55 (78.57)</td>
</tr>
<tr>
<td>10² - 10³⁰</td>
<td>0 (0.0)</td>
<td>16 (22.85)</td>
<td>9 (12.85)</td>
<td>1 (1.42)</td>
<td>14 (20.00)</td>
<td>7 (10.00)</td>
</tr>
<tr>
<td>10³ - 10⁴⁰</td>
<td>2 (2.85)</td>
<td>16 (22.85)</td>
<td>24 (34.28)</td>
<td>23 (32.85)</td>
<td>23 (32.85)</td>
<td>4 (5.71)</td>
</tr>
<tr>
<td>10⁴ - 10⁵⁰</td>
<td>12 (17.14)</td>
<td>12 (17.14)</td>
<td>18 (25.71)</td>
<td>26 (37.14)</td>
<td>17 (24.28)</td>
<td>4 (5.71)</td>
</tr>
<tr>
<td>10⁵ - 10⁶⁰</td>
<td>26 (37.14)</td>
<td>3 (4.28)</td>
<td>13 (18.57)</td>
<td>18 (25.71)</td>
<td>8 (11.42)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>10⁶ - 10⁷⁰</td>
<td>22 (31.42)</td>
<td>0 (0.00)</td>
<td>2 (2.85)</td>
<td>2 (2.85)</td>
<td>5 (7.14)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>10⁷ - 10⁸⁰</td>
<td>8 (11.42)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
</tbody>
</table>

Table I.—Aerobe mesophile plate count, *Pseudomonas*, enterobacteriaceae, enterococci, micrococci/staphylococci, coagulase positive staphylococci distribution (per g) in ground beef.

APC: Aerobe mesophile plate count; CFC: *Pseudomonas*; VG:Enterobacteriaceae; SB: Enterococci; BP: Micrococci/Staphylococci; CPS: Coagulase positive staphylococci

<table>
<thead>
<tr>
<th>MPN / g</th>
<th>Coliform (%)</th>
<th><em>E. coli</em> (%)</th>
<th><em>Salmonella</em> spp. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 (0.00)</td>
<td>49 (70.00)</td>
<td>13 (60.00)</td>
</tr>
<tr>
<td>1 – 3.01</td>
<td>2 (2.85)</td>
<td>1 (1.42)</td>
<td>5 (7.14)</td>
</tr>
<tr>
<td>3.05 – 9.44</td>
<td>1 (1.42)</td>
<td>6 (8.57)</td>
<td>1 (1.42)</td>
</tr>
<tr>
<td>11 – 93.3</td>
<td>6 (8.57)</td>
<td>10 (14.28)</td>
<td>1 (1.42)</td>
</tr>
<tr>
<td>115 – 1100</td>
<td>16 (22.85)</td>
<td>3 (4.28)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>&gt;1100⁰</td>
<td>45 (64.28)</td>
<td>1 (1.42)</td>
<td>0 (0.00)</td>
</tr>
</tbody>
</table>

Table II.—Coliform, *E. coli*, *Salmonella* spp. distribution in ground beef.

*Maximum level detected > 1100 MPN / g.*
about isolation of *E. coli* O157:H7 from ground beef in Turkey. One of them, Noveir et al. (25) reported that *E. coli* O157 was isolated in 0.4% of samples, but none of the isolates of *E. coli* O157 was found to be H7 serotype. Another study, by Akkus (2) reported that no *E. coli* O157:H7 was isolated in 80 minced beef samples. In another study previously conducted by us, we were unable to isolate *E. coli* O157:H7 in ground beef. In another countries, Fantelli and Stephan (13) reported that shigatoxin-producing *E. coli* was isolated from 5/211 (2.3%) minced beef samples. Baumgartner and Grand (5) investigated 166 minced beef samples and found a STEC prevalence of 2.4%. In a study of Svoboda et al. (42) 1.7% of minced meat samples were positive for STEC. In last both studies, however, results on further strain characterisation are lacking.

When microbiological testing is done, it is generally recommended that levels of generic *E. coli* also be assessed as a direct indicator of faecal contamination. Enteric pathogens such as *E. coli* O157:H7 and *Salmonella* are present sporadically and this makes routine monitoring for their presence impractical. In addition, methods for the quantitative recovery of these pathogens are lacking. However, in situations where the level of generic *E. coli* is increased, the risk of these pathogens being present is also considered to be increased. Therefore, the level of generic *E. coli* may be used as indicative of the extent of faecal contamination and hence an indicator of increased potential risk posed to the consumer when a lot of ground beef has been found positive for *E. coli* O157:H7. However, the ratio of levels of different pathogens e.g. *E. coli* O157:H7 versus generic *E. coli* may not be fixed.

In general, staphylococci may be expected to exist, at least in low numbers, in all food products that are of animal origin or in those that are handled directly by humans, unless heat processing steps are applied to effect their destruction [17]. The present study, we found that 42% of all samples contained >10^4 cfu/g micrococci/staphylococci. Coagulase positive staphylococci were detected in 21.4% of samples and 5.7% of the samples contained >10^0 cfu/g. Many authors reported that coagulase positive staphylococci ranged from 10^2 - 10^4/g [21,38] and the isolation ratio was 2.3 - 16.6% [19,21,25]. The study of Minor and Marth (23) revealed that *S. aureus* counts of 10^5-10^6 cells per gram are needed to produce a pathogenic dose of enterotoxin; and the results observed in the present study are not very high. The low count is unable to compete with the normal food-borne bacteria of meat [48]. Staphylococci are commonly found on the skin and in the upper respiratory tract of man and animals and can easily contaminate foods of all forms [14,17]. They produce toxin in the food before it is eaten and cause symptoms that develop rapidly and usually within 24 hours. Though the organisms themselves are readily destroyed at pasteurizing temperatures, the toxin appears to be heat-stable and is not inactivated by boiling or refrigeration for long periods [17].

According to the Turkish regulations, regarding microbiological quality of ground beef; the maximum number of APC must be 5.0x10^5-5.0x10^6 (2 out of 5 samples), *E. coli* 5.0x10^1-5.0x10^2 (2 out of 5 samples), and *E. coli* O157:H7 and *Salmonella* must be absent (n=5), the present study results are very high for APC. *Salmonella* spp., *S. aureus* and *E. coli*.

According to analysis, the microbiological quality of the ground beef analyzed was unsatisfactory, and the product might be an important cause of food poisoning. Turkish people sometimes eat raw ground beef in meatball forms (Turkish name Çiğ köfte). Öz et al., (27) reported in their study that *E. coli* 157:H7 was isolated in 1% of samples of raw meatball. Therefore, prevention measures include warning consumers of the health risks associated with eating raw ground beef and encouraging them to thoroughly cook ground beef and to adhere to safe food handling guidelines.

In conclusion, the results of this study demonstrated that ground beef samples were contaminated with food-borne pathogens bacteria such as *Salmonella* spp., coagulase positive staphylococci and *E. coli*. Further research focusing on effective prevention of food-borne illness is essential for developing intervention and mitigation strategies to reduce the presence of food-borne bacterial pathogens at the retail level. Options for better control include continued improvements in slaughter plant hygiene and control measures under HACCP. In addition to consumer advisories, interventions to reduce the risks associated with the consumption of ground beef include:

1. producers of ground beef to emphasize employee education and training on the recommended methods of cleaning and sanitizing meat-grinding equipment;
2. manufacturers to design meat grinding equipment that is easily accessible for cleaning and sanitization; and
3. state regulatory and inspection authorities to adopt and enforce FDA’s Food Code model requirements, which offer specific recommendation for handling, cooking, and storing raw meat cleaning and sanitizing equipment and utensils; designing and constructing equipment; and advising consumers about the risks associated with consumption of raw or undercooked food of animal origin.

**References**


