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Research Article

Presence and Contamination Level of *Clostridium perfringens* in Raw Frozen Ground Poultry and Poultry Burgers

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Abstract: The objective of this study was to determine the occurrence and the enumeration of *Clostridium perfringens* in ground poultry and burgers using the MPN technique and selected biochemical tests, including acid phosphatase and reverse-CAMP tests. Forty raw frozen ground poultry and 40 frozen poultry burger samples were purchased from different poultry processing plants in Turkey, between June and December 2000. The samples were taken aseptically and transported to the laboratory in refrigerated containers and tested the same day.

C. perfringens was isolated from 28 (70.0%) of the 40 ground poultry samples at the mean number of 2.6 MPN/g. The minimal and maximal numbers of ground poultry samples positive for *C. perfringens* varied from 0.30 to 9.3 MPN/g. Only 1 (2.5%) of the 40 poultry burger samples was positive for *C. perfringes* at the mean number of 0.36 MPN/g. *C. perfringens* was found higher in the warm months of July to early September (67.9%) than in October to late December (32.1%) in 28 positive poultry ground samples. Only one poultry burger sample positive for *C. perfringens* was taken in July.

In conclusion, ground poultry may be considered a significant source of *C. perfringens* mainly in warm months. The high incidence of this bacterium in ground poultry may indicate insanitary conditions and improper handling at processing plants.

Key Words: Ground poultry, poultry burger, *Clostridium perfringens*, MPN

Donmuş Çiğ Tavuk Kıyma ve Burgerlerde *Clostridium perfringens*'in Varlığı ve Kontaminasyon Düzeyi

Özet: Bu çalışmada tavuk etinden yapılan kıyma ve burgerlerde *Clostridium perfringens*'in varlığının ve kontaminasyon düzeyinin MPN tekniği ve asit fosfataz, reverse-CAMP gibi biyokimyasal testler kullanılarak saptanması amaçlanmıştır. Çalışmada, Haziran-Aralık 2000'de farklı kanatlı kesimhanelerinden alınan 40 donmuş tavuk kıyması ve 40 tavuk burger örneği materyal olarak kullanılmıştır. Aseptik koşullar altında alınan örnekler soğuk zincir altında laboratuara getirilerek, aynı gün içinde analizlere başlanmıştır.

İncelenen 40 tavuk kıyması örneğinin 28'inden (% 70,0) ortalama 2,6 MPN/g düzeyinde, 40 tavuk burger örneğinin yalnızca 1'inden (% 2,5) 0,36 MPN/g düzeyinde *C. perfringens* izole edilmiştir. Toplam 28 pozitif tavuk kıyması örneğinde *C. perfringens* izolasyon oranı Haziran- Eylül başına kadar olan sıcak aylarda % 67,9 ile, Ekim-Aralık sonuna kadar olan soğuk aylardan (% 32,1) daha yüksek bulunmuştur. *C. perfringens* izole edilen tek tavuk burger örneği Haziran ayında alınmıştır.

Sonuç olarak bu çalışmada incelenen donmuş tavuk kıymalarının özellikle sıcak yaz aylarında *C. perfringens* yönünden önemli bir kaynak olduğu saptanmıştır. Bu bakterinin tavuk kıymasındaki yüksek insidensi göz önünde bulundurularak güvenli kanatlı eti ve ürünleri üretilmesi için işletmelerde gerekli hijyenik önlemlerin alınması önerilmiştir.

Anahtar Sözcükler: Tavuk kıyma, tavuk burger, *Clostridium perfringens*, MPN

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Introduction

Clostridium perfringens is an anaerobic, spore-forming and ubiquitous pathogen bacterium widely distributed in the environment and frequently occurs in soil, water and the intestinal tract of certain animals and humans. *C. perfringens* is the common causative agent of classic type A diarrhea but more rare type C human necrotic enteritis. Illness occurs due to consumption of food contaminated with large numbers ($>10^6$) of viable vegetative cells of *C. perfringens* per gram of implicated food, followed by sporulation and enterotoxin (CPE) formation in the small intestine (1-4). The production of the enterotoxins is the main virulence factor of common form of foodborne disease. Among the 13 different types of toxins known produced by this organism is *C. perfringens* enterotoxin, which is a single polypeptide of 35 kDa with a unique amino acid sequence (5).

Numerous epidemiological investigations have revealed that the majority of foodborne outbreaks are associated with the consumption of meat and poultry products. The incidence is widely under-reported due to the perceived mild nature of the illness and differences between countries in their surveillance arrangements. Foodborne diseases are more likely to occur where contaminated foods are re-cooked or kept warm for long periods of time (6). Most outbreaks involving *C. perfringens* are reported from institutional catering such as restaurants, factories, hospitals, schools and caterers mainly for elderly people (5,7).

C. perfringens was reported as a contaminant of the processing plant and processed carcasses of broiler chickens. Following slaughter the broilers must undergo scalding, defeathering, removal of offal and washing, and cutting of ground meat, which provide the contamination of the carcasses primarily from intestinal contents of animal during the slaughter process via cross contamination. Therefore reports revealed a high incidence of *C. perfringens* in poultry (8-10). After the grinding procedure of poultry meat there are increases in the incidence and the level of contamination of ground poultry by *C. perfringens*. Poultry meat is minced and used for the production of raw poultry products including poultry meatballs and burgers as well as heat-processed products in many countries.

Few studies have been published on the quantity of *C. perfringens* in poultry products such as ground poultry and poultry burgers. Therefore, the objective of this study was to determine the occurrence and the enumeration of *C. perfringens* using the MPN technique and selected biochemical tests, including acid phosphatase and reverse-CAMP tests.

Materials and Methods

Forty raw frozen ground chicken and 40 ready-to-cook frozen chicken burger samples were purchased from 6 different poultry processing plants located in different regions in Turkey, between June and December 2000. The samples were taken aseptically and transported to the laboratory in refrigerated containers and tested the same day.

The techniques described by Labbe (2), Baumgart et al. (11), and Schalch et al. (12) were used to isolate and identify *C. perfringens.* The level of contamination of analyzed samples with *C. perfringens* was determined by the MPN (Most Probable Number) technique (2). For enrichment and MPN-determination (3 tubes) of *C. perfringens* a 25-g portion of each sample was aseptically placed in a sterile plastic bag containing 225 ml of Perfringens Enrichment Medium (PEM; Fluid Thioglycollate Medium, supplemented with Perfringens (TSC) supplement, Oxoid SR 88, Oxoid, UK) and homogenized by a stomacher (Colworth Stomacher 400, UK) for approx. 2 min. Ten milliliters of these homogenates were transferred into 3 tubes, and 1 and 0.1 ml each of these homogenates were also added to 3 tubes containing 9 and 10 ml of PEM broth, respectively. The tubes were overlaid with sterile melted paraffin to create an anaerobic environment and then incubated at 46 °C for 20 h without agitation. After the samples were enriched in PEM, one loopful from each tube that produced gas and turbidity was streaked onto TSC agar (Tryptose Sulphite Cycloserine agar, Oxoid CM 857, Oxoid, UK) and the plates were further incubated at 46 °C for 20 h in a Gas Pak system (Gas generating kit, B 36, Oxoid) anaerobically. In order to confirm of *C. perfringens*, up to 5 suspect black colonies from each positive TSC agar plate were purified and identified biochemically by using catalase test, lactose fermentation, gelatinase production, nitrate reduction, motility test, acid phosphatase reaction, haemolysis test and the reverse CAMP-test.

The MPN counts of *C. perfringens* were estimated according to the MPN table (13).

The pH values of the samples were measured by a digital pH-meter (Nel Electronic, TR).

Results

The results of this study are given in Table. *C. perfringens* was isolated from 28 (70.0%) of the 40 ground poultry samples at the mean number of 2.6 MPN/g. The minimal and maximal numbers of ground poultry samples positive for *C. perfringens* varied from 0.30 to 9.3 MPN/g (data is not shown in the table). The high incidence of *C. perfringens* in the ground poultry samples tested may be explained by the several processing stages from slaughter to final grinding at slaughterhouse level. Only 1 (2.5%) of the 40 poultry burger samples was positive for *C. perfringes,* at the mean number of 0.36 MPN/g. *C. perfringens* was higher in the warm months of July to early September (67.9%) than in October to late December (32.1%) in 28 positive poultry ground samples. Only one poultry burger sample positive for *C. perfringens* was taken in July.

All isolates tested and confirmed by standard biochemical tests showed a positive reaction to the acid phosphatase and reverse-CAMP tests. These tests proved to be sensitive, rapid and reliable for the identification of *C. perfringens.*

Mean pH values of ground poultry and burger samples positive for *C. perfringens* were 6.6 and 6.1, respectively.

Discussion

In recent decades many surveys have been conducted on the incidence of *C. perfringens* in raw and processed meat and poultry. These reports indicate widespread occurrence of the organism in meat and poultry (2,5).

Our results are in agreement with those published by Craven (14), who reported the incidence of *C. perfringens* in poultry carcass rinse samples as 67%. Similarly Miwa et al. (10) detected *C. perfringens* in 42 (84%) of 50 chicken samples in Japan. In the presence of enteropathogenes in the intestines of broilers, the carcass can become contaminated with the microorganisms during the slaughter process, which results in the contamination of the end products. It has been reported that the incidence of *C. perfringens* in the intestinal contents of chicken was higher than of cattle and swine (9).

In contrast to this and previous other studies, low incidence was reported by other researchers: Lin and Labbe (15) found this bacterium in 39 (29.5%) of the 132 food samples including chicken meat, chicken leg and chicken neck obtained from retail outlets in Western Massachusetts in the USA. None of the isolates was able to produce CPE. Saito (4) isolated *C. perfringens* from 16 (24%) of 68 chicken meat samples and none of the strains was positive for CPE. The author found the highest level of *C. perfringens* in professional food handlers. Most surveys of the incidence of *C. perfringens* have not identified the enterotoxin-producing potential of the isolates.

The prevalence of *C. perfringes* in ground poultry samples tested in the present study was higher than that reported in previous studies by Lin and Labbe (15) and Saito (4). The higher prevalence may be the result of different sampling regimes and isolation techniques used in the various studies, and differences in the hygienic conditions of the processing plants and product.

The relative low number of *C. perfringens* found by the MPN technique in our study is in good agreement with findings reported by other researchers. Craven (14) reported the mean number of *C. perfringens* in poultry carcass rinse samples as MPN $\log_{10}$ 1.20 per/100 ml. Similarly Lin and Labbe (15) found that chicken samples

Table. Occurrence and enumeration of *Clostridium perfringens* in poultry samples.

Number of the samples tested		Number of the samples positive for *C. perfringens* (%)	Mean number of *C. perfringens* in positive samples (MPN/g)
Ground poultry	40	28 (70.0)	2.6
Poultry burger	40	1 (2.5)	0.3

including chicken, chicken leg and chicken neck samples contained *C. perfringens* at levels between 3.05 and >1.100 MPN/g. Among the positive samples a chicken leg sample had the highest level of *C. perfringens*. Furthermore, Miwa et al. (10) detected *C. perfringens* in chicken samples at the levels of $<10^2$ and 10^4 MPN/100 g (25 samples at $<10^2$, 11 samples 10^2-10^3 and 6 samples 10^3-10^4 MPN/100 g).

This study showed that the incidence of *C. perfringens* was higher in the warm months, with the rate of 67.9%. Krause et al. (16) isolated sulfite reducing *Clostridium* in 90% of ground beef samples collected from June to July in Ankara, Turkey.

There is no published literature on the incidence of *C. perfringens* in poultry burgers, which are made from ground poultry. In the present study, *C. perfringens* could be isolated only from 1 of the 40 poultry burger samples. The higher incidence (70.0%) of this bacterium in ground poultry samples tested may be explained by the fact that poultry burgers are produced by adding ingredients such as salt, sodium lactate, sodium nitrite, sodium propionate and sorbic acid, which inhibit the growth and sporulation of *C. perfringens*.

Salts of organic acids such as sodium or potassium lactate and sodium diacetate are extensively used in meat and poultry products to enhance the microbiological safety of these products by controlling foodborne pathogens. A report by Juneja and Thippareddi, (17) also indicated that sodium lactate, sodium acetate, buffered sodium citrate (Ional^{TM}) or buffered sodium citrate supplemented with sodium diacetate (Ional Plus^{TM}) at 1% concentration substantially inhibited the germination and outgrowth of *C. perfringens* spores in marinated ground turkey breast.

Kalinowski and Tompkin (18) reported that the growth of the psychrotrophic Clostridia was inhibited in a broth media by 3.0% NaCl, 100 ppm nitrite, 2.0% sodium lactate and 0.2% sodium diacetate. Taormina et al. (19) showed that processed meat products cured with sodium nitrite are not at risk for the growth of *C. perfringens* during extended chilling and cold storage.

Sabah et al. (20) also found that sodium citrate, sodium lactate and sodium diacetate added to a beef system were able to decrease the number of *C. perfringens* about 1 log. Thippareddi et al. (21) reported the reduction of *C. perfringens* in a roast beef formulation from 0.98 to 2.47 log by adding of buffered sodium citrate at the levels of 0.5-2.0%. In cook-in-bag turkey breast formulation the combination of sodium lactate (2.5%) and sodium diacetate (0.25%) inhibited effectively the growth of *C. perfringens* and prolonged the shelf life (22). Aran (23) reported similarly that the adding of 1.5% of sodium lactate inhibited the growth of *C. perfringens* at all storage temperatures in a sous-vide goulash. The heat resistance of *C. perfringens* was inhibited by 0.3% sodium pyrophosphate (24).

Various media and methods have been developed recently for more effective isolation and enumeration of this bacterium from food. In addition to the standard biochemical tests the combination of acid phosphatase and reverse-CAMP tests was also used in this study. In agreement with De Guzman et al. (25) and Eisgruber et al. (26), the use of acid phosphatase and reverse-CAMP tests provided reliable results for the confirmation of *C. perfringens*.

In conclusion, ground poultry may be considered a significant source of *C. perfringens* mainly in warm months. The high incidence of this bacterium in ground poultry may indicate insanitary conditions and improper handling at processing plants. GMP and the implementation of the HACCP system at poultry processing plants are major contributing factors to produce safe poultry products and to avoid public health problems.

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