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Detection of *Clostridium perfringens* Contamination in Retail Minced Beef and Poultry Meat Retailed in Afyon*

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Summary: The aim of the present study was to determine the prevalence and contamination level of *Clostridium perfringens* (*Cl.perfringens*) in minced poultry and beef sold at retail in Afyon. The contamination level of *Cl. perfringens* was detected by Most Probable Number (MPN) method. A total of 100 samples, consisting of 50 raw minced poultry meat, and 50 minced beef were collected from various retail stores and butchers. Our results demonstrated that 61 of 100 saples (61%) were contaminated with between 3.0 and 20.0 MPN/g *Cl. perfringens*. Thirty four of 50 minced beef samples (68%) and 27 out of 50 minced poultry meat samples (54%) were found to be contaminated with *Cl. perfringens*. Mean contamination level for minced beef and poltry meat samples were 4.9 MPN/g and 3.5 MPN/g, respectively. In our study, it was determined that the increase in air temperature results in increase in *Cl. perfringens* prevalenceand contamination level of the samples collected from were higher than those collected from supermarket.

Key Words: Beef, *Clostridium perfringens*, poultry meat

Afyon'da Satışa Sunulan Sığır ve Tavuk Kıymalarında *Clostridium perfringens* Kontaminasyon Düzeylerinin Belirlenmesi

Özet: Bu çalışmada, Afyon'da süpermarket ve kasaplarda satışa sunulan tavuk ve sığır eti kıymalarında *Clostridium perfringens*'in (*Cl. perfringens*) prevalansı ve kontaminasyon düzeyininin belirlenmesi amaçlandı. Kontaminasyon düzeyi EMS (En Muhtemel Sayı) yöntemi ile belirlendi. Farklı kasap ve satış yerlerinden toplam 100 örnek (50 tavuk kıyma; 50 sığır kıyma) toplandı. Çalışmanın sonuçları 100 örneğin 61'inin (%61) 3.0-20.0 EMS/g düzeyleri arasında kontamine olduğunu gösterdi. Sığır kıyma örneklerinin 34'ü (%68), tavuk kıyma örneklerinin ise 27'si (%54) *Cl. perfringens* ile kontamine bulundu. Sığır kıyma ve tavuk kıyma örneklerinin sırasıyla ortalama 4.9 EMS/g ve 3.5 EMS/g düzeylerinde kontamine olduğu belirlendi. Artan hava sıcaklığına bağlı olarak prevalansın ve kontaminasyon düzeyininde arttığı, kasaplardan toplanan örneklerin, süpermarketlerden toplananlara göre daha kontamine olduğu belirlendi.

Anahtar Kelimeler: *Clostridium perfringens*, kanatlı eti, sığır eti

Introduction

Clostridium perfringens is a gram-positive, non-motil, rod, sporulated and is a widely distributed bacteria in the environment and normal intestinal flora of humans and animals. Since the first large-scale outbreaks of food poisoning associated with this organism reported by Knox and MacDonald in 1943 (21), the organism has been responsible for foodborne poisoning in the industrialized world (36). The organism is a major cause of human foodborne disease, and an important pathogen of human gastrointestinal (GI) tract diseases such as food poisoning, antibiotic-associated diarrhea, and sporadic diarrhea as well as nosocomial diarrheal disease outbreaks (27, 28). Most pathogenic *Clostridium* species are known to produce toxins, which are responsible for a wide range of diseases (17). An estimated 9.4 million cases of foodborne disease occur each year in the United States and 1 million (10%) of these cases have been found to be the result of *Cl. perfringens* poisoning (36). Deaths from *Cl. perfringens* type A food poisoning are not common but do occur in the elderly and debilitated. Per year this food poisoning is

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estimated to kill seven people in the United States and between 50 and 100 people in the United Kingdom (1, 30).

Foodborne illness as occur after the ingestion of food contaminated with a large number (10^6–10^7 cells/g) of type A viable vegetative *Cl. perfringens* cells. *Cl. perfringens* enterotoxin (CPE) are produced during *in vivo* sporulation, which usually occurs in the small intestine and is stimulated by acid conditions (5, 29). Approximately 8 to 12 h after eating the contaminated food, the symptoms start with acute abdominal pain, nausea and diarrhea. The contaminated food is almost always heat-treated, which kills competing flora, while the *Cl. perfringens* spores survive and germinate (6, 20). This organism has two main characteristics that contribute to its ability to cause foodborne diseases. Firstly, its low generation time (reportedly <10 min for vegetative cells) allows *Cl. perfringens* to quickly multiply in foods (28, 38), and secondly its relatively higher heat tolerance enhances its ability to survive in undercooked foods. Generally, *Cl. perfringens* is a difficult target bacterium for microbial source tracking because it is so broadly distributed in the environment (18, 34). However, only a small proportion of isolates of this bacterium can produce toxins specific for human and animal gastrointestinal diseases (7, 19, 23). Type A strains have been found in 5% of human fecal samples or environmental sources such as nonoutbreak retail foods (26, 31, 42).

All *Cl. perfringens* food poisoning outbreaks have been caused by strains type (A) for which meat is an excellent growth medium (3). *Cl. perfringens* requires more than a dozen amino acids and several vitamins for its growth, both of which are typically present in meat. Recent Central for Diseases Control and Prevention (CDC) statistics indicate that the leading food vehicles for this bacterium in the United States are meats, notably beef and poultry, and meat-containing products, such as gravies, stews, and Mexican food (24, 28).

The aim of the present study was to determine the prevalence and contamination level of *Cl. perfringens* in minced poultry and beef sold at retail markets.

Material and Methods

Collection of food samples

Fifty raw minced poultry and 50 minced beef samples were purchased from different butchers and supermarkets in Afyon between April and July 2011. Total of 100 samples (33 from buthchers and 67 from supermarkets) (Table 2). All of minced poultry meat were obtained from poultry breast meat and all of minced meat were obtained from lobes meat. The samples were taken aseptically and transported to the laboratory in refrigerated containers and tested within 1-2 h of sampling. The Most Probable Number (MPN) counts of *Cl. perfringens* were estimated according to the MPN table (13).

Determination of the MPN of Cl. perfringens bacteria per gram in samples

The techniques described by Labbe (1989), Baumgart et al. (1990), and Schalch et al. (1996) (4, 22, 37) were used to isolate and identify *Cl. perfringens*. The contamination level of analyzed samples with *Cl. perfringens* was determined by the MPN (Most Probable Number) technique (22). For enrichment and MPN-determination (3 tubes) of *Cl. 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 approximately 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^{o}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 Tryptose Sulphite Cycloserine agar (TSC agar, Oxoid CM 857, Oxoid, UK) and the plates were further incubated at 46^{o}C for 20 h in a Gas Pak system (Gas generating kit, B 36, Oxoid) anaerobically. In order to confirm the isolates, 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.

Statistical Analysis

Chi square test was used to evaluate the results.

Results

Of the 100 samples tested, 61 (61%) were found to be contaminated with *Cl*. *perfringens* and a total of 61 strains were isolated (Table 1). All samples showed *Cl*. *perfringens* contamination rates ranging from 68% (beef) to 54% (minced poultry meat) (Table 1). Most of the samples surveyed in this study had very high MPN per-gram values higher than 3 (average 4.9 MPN/g) (Table 1). The samples were found to be contamineted with *Cl. perfringens* between the levels of 3.0 and 20.0 MPN/g. In this study, minced beef and poltry meat samples were found to be contaminated with *Cl. perfringens* at average of 4.9 MPN/g and 3.5 MPN/ g, respectively.

According to the results, the contamination level of the samples collected from butchers found to be higher than that collected from the supermarkets. It has been observed that butchers are more unfavorable in terms of hygienic conditions and rules. Prevalance of *Cl. perfringes* in different sampling places are summerized in Table 2. With an increase in air temperature, *Cl. perfringens* was seen to increase in prevalence and contamination level. Prevalence of *Cl. perfringens* increased steadily from April to July as seen in Table 3.

Discussion

Various results are reported concerning the presence of *Cl. perfringens* in food, human feces, and the environment (7, 19, 23, 26, 31, 42). Meat and poultry products have been implicated in numerous outbreaks of foodborne disease associated with the organism (14). It is prevalent in

Table 1. Incidence of *Cl. perfringens* in different samples.

Food	No. of samples examined	*No. (%) of samples contaminated with Cl. perfringens*	MPN/g average range
Minced beef	50	34 (68%)	4.9-20
Minced poultry meat	50	27 (54%)	3.5-20
Total	100	61 (61%)	4.2

Table 2. Prevalance of *Cl. Perfringens* in different sampling places.

		Cl. perfringens		Total	Value of p
		(+)	(-)		
Butcher	(n)	21	12	33	**0.704**
	(%)	63.6	36.4	100	
Supermarket	(n)	40	27	67	
	(%)	59.7	40.3	100	
Total	(n)	61	39	100	
	(%)	61.0	39.0	100	

(n) :number of samples
Value of p : Value of significance

Table 3. Prevalance of *Cl. Perfringens* and distributions in months.

		Cl. perfringens		Total	*Value of p*
		(+)	(-)		
April	(n)	6	9	15	**0.097**
	(%)	40.0	60.0	100.0	
May	(n)	19	16	35	
	(%)	54.3	45.7	100.0	
June	(n)	17	8	25	
	(%)	68.0	32.0	100.0	
July	(n)	19	6	25	
	(%)	76.0	24.0	100.0	
Total	(n)	61	39	100	
	(%)	61.0	39.0	100.0	

(n) : number of samples
Value of p : value of significance

broiler chicken operations; isolation rates from processed chicken carcasses range from 8 to 68% (11, 12). Animal carcasses and cuts of meat can become contaminated with *Cl. perfringens* from contact with soil or animal feces, or during slaughtering and processing (8). Many organisms that compete with *Cl. perfringens* are killed when meat and poultry are cooked, but *Cl. perfringens* spores are difficult to eliminate (15).

Minced meat is a very common meat product in Turkey. Meat when turned into minced meat, there are no natural barriers of muscle tissue and microorganisms that may spread to each side of the meat. Cell fluid of the meat prepare an environment suitable for the development of bacterial reproduce and growth.

Contamination of meat and meat products with *Cl. perfringens* may be through different sources; mainly internally from animal after slaughter as postmortem invasion or externally from contaminated hands, skin of animals, soil, water and processing equipments (35).

In this study, the incidence of *Cl. perfringens* in minced beef (68%) was high in comparison with minced poultry meat (54%). *Cl. perfringens* was found in 61% (average 4.9 MPN/g) of samples. Similarly, *Cl. perfringens* was isolated from 88% of chicken carcasses consumed in Beijing (16); and 70.4% of chicken meat collected from slaughterhouses and markets in India (39). An earlier survey by Wen and McClane (42) reported that 56 of 147 chicken samples (%38), 17 of 83 (%21) cattle meat samples, and 25 of 108 (%23) minced beef samples at 2-5 MPN/g, 1-10 MPN/g, 3-32 MPN/g. In addition, it is reported that in 84% out of 50 chicken samples, 16% beef samples in Japan were contaminated with *Cl. perfringens* (32). *Cl. perfringens* were isolated from 24% of minced meat consumed in *San Luis* (40), 70.4% of chicken meat collected from slaughterhouses and markets in India (39), 66% of wing and chicken leg quarter samples in Canada (33), and 8.7-29.6% out of 150 minced beef samples in Casablanca (8). In present study, it was observed that the samples were contamineted with *Cl. perfringens* between the levels of 3.0 and 20.0 MPN/g, while Craven (10), Lin and Labbe (26), and Miwa et al. (32) found 1.20 MPN $\log_{10}$ in chicken carcasses, 3.05 and >1.100 MPN/g in chicken meat parts, $<10^2$ ve 10^4 MPN/100g in poultry meat respectively.

Generally, the contamination level of poultry meat samples was higher than that of cattle meat samples in other studies. In this study, the contamination level of beef samples was higher than that of poultry samples. This situation could be due to increased contamination risk of the

cattle carcass during evisceration as the organism is natural flora of the gut and soil. Traditionally, food raw materials have been considered to be the main source of food contamination with *Cl. perfringens*. The high prevalence of toxin positive strains in the human gastrointestinal tract may serve as an important source of contamination for healthy people handling foods or food raw materials (19). On the other hand, it can be related to stress from shipping and different management conditions prior to arrival such as hygiene, population, stocking density along with potential geographic variation and differences in methodology (9). Overall, the findings in regard to *Cl. perfringens* (54% positive) are very similar to those in other studies that examined retail poultry meat, where the positivity rate ranged from 26 to 98% (26, 31). It is not surprising that, in this study results show that a high number of retail minced poultry meats are positive for *Cl. perfringens*. The presence of this enteropathogen in the intestines of birds can contaminate the final product. Foods of animal origin, which are rich in protein have great importance in the occurrence of food poisoning caused by *Cl. perfringens*. Factors are widely available in the products of raw meat and raw or inadequately cooked meats.

Cl. perfringens were isolated from 88% of chicken carcasses consumed in Beijing (16), 70.4% of chicken meat in India (39) and 66% of wing and chicken leg quarter samples in Canada (33). However, in a study conducted in different retail stores in the United States, it was reported that 4 (21%) of 19 chicken meat samples were contaminated with *Cl. perfringens*. In another study where samples were collected from different broiler chicken slaughterhouses in Sweden, 114 (18%) out of 636 chicken carcasses were determined to be contaminated with *Cl. perfringens* (25, 26). The reasons for the differences between the research findings are thought to be due to the technological differences between the slaughterhouses and different methodology used. High rate of prevalence in the chicken meat can also be attributed to: i) the bacterium being ubiquitous and the availability of poultry intestinal flora, ii) the inevitability of cross-contamination due to the poultry slaughter process, and iii) the lack of sanitation and hygiene conditions (2, 41).

Conclusion

The result of the present study highlighted that *Cl. perfringens* contamination rates in raw poultry and beef minced meat and its products in Tukey may constitute a health hazard to consumers, especially to people at greater risk.

Products, especially minced meat is gaining popularity in Turkey as they represent easily prepared meals. Cooking should be done under ideal conditions. Efforts to reduce illnesses from *Cl. perfringens* in ready-to-eat and partially-cooked meat and poultry products should focus on retail and consumer storage and preparation methods. Besides, proper control strategies are required to reduce the number of disease cases and outbreaks frequently caused by this organism.

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