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Sığır eti

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Determination and Vital Activity of Thermophilic *Campylobacter* (*Campylobacter Jejuni*) In The Turkish Pastırma and Turkish Style Fermented Sausage (Sucuk) Commercially Consumed In Market and Experimentally Produced With Inoculation Of Different Levels of *Campylobacter Jejuni*

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S U M M A R Y

In this study, Turkish fermented sucuk (n=30) and Turkish pastırma (n=30) samples sold in retail stores were analyzed in terms of *C. jejuni*. Afterwards, *C. jejuni* was experimentally inoculated at different levels (Group I:10^2, Group II:10^4, and Group III:10^6 cfu/ml) into the mixtures of traditional Turkish fermented sucuk and çemen (spice mixture of Turkish pastırma) during the process. *C. jejuni* was monitored bacteriologically during fifteen days of fermentation. *C. jejuni* was not detected in the samples of Turkish fermented sucuk (n: 30), Turkish pastırma (n: 30), and raw beef meat samples purchased from the retail markets for use in manufacturing. The numbers of *C. jejuni* were below the detection level of 10^2 cfu/g in all three experimental Turkish fermented sucuk groups at the end of second day at pH 5.32, and also *C. jejuni* was not detected by Most Probable Number (MPN) technique (<0.30MPN/g) in the same samples. Likewise, *C. jejuni* numbers were below the detection level of 10^2 cfu/g at the end of fourth day at pH 5.19 in the samples of all three experimental Turkish pastırma groups, and also *C. jejuni* was not detected by Most Probable Number (MPN) technique (<0.30MPN/g) in the same samples. In conclusion, the risk of *C. jejuni* infection through consuming traditional Turkish fermented sucuk and pastırma seems to be very low unless cross contamination occurs.

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Piyasada Tüketime Sunulan ve Deneysel Olarak Farklı Düzeylerde *Campylobacter jejuni* İnokule Edilerek Üretilen Türk Fermente Sucuğu ve Pastırmalardaki Termofilik *Campylobacter* (*Campylobacter jejuni*) Varlığı ve Yaşamsal Aktivasyonu

ÖZET

Bu araştırmada, piyasada tüketime sunulan 30 adet Türk fermente sucuğu ve 30 adet Türk pastırması *C. jejuni* bakımından analiz edildi. Takiben, üretim aşamasında geleneksel Türk fermente sucuğunun hamuruna ve Türk pastırmasının çemenine *C. jejuni* deneysel olarak farklı gruplarda inoküle edildi. (Grup I:10^2, Grup II:10^4, ve Grup III:10^6 kob/ml). On beş gün süren fermentasyon süresince *C. jejuni* düzeyi incelendi. Piyasadan toplanan 30 adet Türk fermente sucuğu ve 30 adet Türk pastırması ve marketlerden üretimde kullanılmak amacıyla temin edilen çiğ sığır etlerinde *C. jejuni* saptanmadı. Deneysel olarak üretilen Türk fermente sucuklarında pH 5.32'de 2. günün sonunda her 3 grupta *C. jejuni* düzeyi 10^2 cfu/g'ın altında saptandı. Aynı örneklerde, *C. jejuni* düzeyi En Kuvvetle Muhtemel Sayı Tekniği ile (EMS) (<0.30MPN/g) olarak saptandı. Benzer biçimde, deneysel olarak üretilen Türk pastırmalarında *C. jejuni* düzeyi her 3 grupta 4. günün sonunda pH 5.19'da 10^2 cfu/g'in altında saptandı. Aynı örneklerde *C. jejuni* düzeyi En Kuvvetle Muhtemel Sayı Tekniği ile (EMS) (<0.30MPN/g) olarak saptandı. Sonuç olarak, *C. jejuni* infeksiyonunun çapraz kontaminasyonlar olmadığı sürece Türk fermente sucuğu ve Türk pastırmasında çok düşük düzeyde risk yaratabileceği sonucuna varıldı.

INTRODUCTION

Turkish pastırma and Turkish fermented sausage (Sucuk) are consumed all over the Turkey, produced using traditional methods in several cities particularly Kayseri and Afyonkarahisar, Although ingredients like garlic, different spices, salt, nitrite or nitrate combinations, decreasing humidity and water activity in the Turkish pastırma and Turkish fermented sucuk production (especially fermentation process) are the essential factors for lowering the presence of risk factors in both meat products in terms of food borne microorganisms, Since no heat treatment is applied during their production processes. Futher more, these products are also consumed raw which could constitute a health risk. *Campylobacter jejuni* has a minimal infection dose (MID) of $5.0x10^2$ cfu/g (Black et al 1988). Although it possesses very weak competitive characteristics in nature (Griffiths and Park 1990), it has been isolated from red meat (Elmalı 2001, Grau 1998, Little et al 2008, Stern 1981, Stern et al 1984, Stern et al 1985), broiler meat (Atabay and Corry 1997, Elmalı and Yaman 2004, James et al 2007, Kwiatek et al 1990), milk (Kalman et al 2000, Yaman and Elmalı 2004), and fish and water (Yaman et al 2005) in epidemiological studies.

In this study, Turkish fermented sucuk (n=30) and Turkish pastırma (n=30) samples sold in retail stores were analyzed in terms of *C. jejuni*. Afterwards, *C. jejuni* was experimentally inoculated at different levels (10^2, 10^4, and 10^6 cfu/ml) into the mixtures of traditional Turkish fermented sucuk and çemen (spice mixture of Turkish pastırma) during the process. Doing that, this study aimed to monitor the presence of *C. jejuni* in the retail samples and detect the survival of different inoculation numbers of *C. jejuni* during the ripening period of raw traditional Turkish fermented sucuk and processing of Turkish pastırma in terms of risk potential of *C. jejuni* for public health since these products are especially consumed raw as well as cooked.

MATERIAL AND METHODS

Samples:

Thirty traditional Turkish fermented sucuk and thirty Turkish pastırma samples were randomly collected from retail stores, and markeds stored at 4ºC until analyzed.

Experimental culture:

C. jejuni strain was grown under microaerophilic conditions using Campylobacter Enrichment Broth (CEB) and inoculated at different levels (10^2, 10^4, and 10^6 cfu/ml) into the experimentally traditional Turkish fermented sucuk and pastırma çemen mixtures. *C. jejuni* strain used experimentally in this research was isolated and identified from the previous research of Elmalı (2001).

Making traditional Turkish fermented sausage (Sucuk):

Turkish fermented sucuk production was experimentally made according to the Turkish Standards Institute (TSE 1070) (Anon 2002) and the Meat and Fish Office (EBK) (Anon 1988). No *C. jejuni* was detected in the microbiological analysis of raw beef meat used for making Turkish fermented sucuk and Turkish pastırma. Initially, 3.250 kg red cattle meat with less fat was minced in a meat grinder (Tefal). The temperature was below 4ºC during this process. Subsequently, 500 g grinded frozen renal fat, spice content (20 g red pepper+32 g cumin+20 g black pepper), garlic (50 g) and saccharose (20 g) were added to the minced meat. Finally, nitrite-salt conbination (500 mg sodium nitrite-95 g salt) was also mixed into the sucuk mixture. This mixture was divided into three parts to form experimental groups of I, II and III which were experimentally inoculated at different levels of 10^2, 10^4, and 10^6 cfu/ml with *C. jejuni* strain, respectively. Then, sucuk coils, placed in 1% water solution of lactic acid-salt combination, was filled up with these inoculated mixtures using a sucuk making machine (Tefal). Finally, these filled sucuk were rinsed with cold water and stored at 16-20ºC for 15 days for fermentation and drying in the room conditions. Rinsing was carried out for 5 min for three days after filling. Unlike industrial fermented sausage technology, starter culture is not used in the processing of traditional Turkish fermented sucuk in the most of Anatolian cities. Similarly, No starter culture was used for making experimental sucuk in this study.

Making traditional Turkish pastırma:

Turkish pastırma production was experimentally made according to Elmalı et al (2005). Raw beef meat pieces (5 kg) were purchased from a supermarket. Spices, fenu-greek (*Trigonella foenum graceum*), garlic and powdered red pepper used in the çemen mixture (spice paste) were bought from a market in Kayseri, Turkey. To prepare meat pieces, excessive fat and nerves were taken away and removed initially. Then, 1 cm of deeply parallel cuts were conducted on both sides of the meat pieces. An amount of 125 ppm sodium nitrite was added to the salt, After that, at an amount of 5% salt of the

weight of meat, was distributed evenly onto the surfaces of meat pieces. Then after keeping at +4°C for 24 h., the excesive water of meat pieces was removed. Then, The salted meat pieces were turned upside down and were salted second time using the same amount of salt. Following 24 h resting at +4 °C, the salted meat pieces were rinsed again under cold tap water for 1 h to remove excessive salt. Subsequently, the meat pieces were placed in an apparatus designed particularly for pastırma production. The meat pieces were squeezed between two marble layers +4 °C for 48 h using the apparatus to remove excessive water and to obtain a firm product. Following, they were dried in a well ventilated room by hanging on a metal bar for ten days. After that, they were squeezed second time in the apparatus at +4 °C for 24 h and dried again in the same condition. Then, the dried meat pieces were marinated in the çemen mixture containing 50% water, 15% fenu-greek (*Trigonella foenum graceum*), 20% garlic, and 15% red pepper powder, for 2 h as indicated by Dogruer et al (1998). During the process, the pastırma çemen mixture was divided into three parts to form experimental çemen groups of I, II and III which were experimentally inoculated with *C. jejuni* at the levels of 10^2, 10^4, and 10^6 cfu/ml, respectively. Finally, the marinated meat pieces were divided into three groups of I, II and III. Then, each group was embeded into the corresponding experimentally inoculated çemen mixture. These final experimental pastırma samples were dried for 15 days at room temperature.

Isolation and Identification of *C. jejuni*:

Isolation and identification of the *C. jejuni* were performed according to the Food and Drug Administration (FDA) (Anon 1998). In order to isolate *C. jejuni*, each 25 g Turkish fermented sucuk and Turkish pastırma samples were added into 225 ml Campylobacter Enrichment Broth (CEB). They were initially incubated at 37°C for 2-4 hours and then at 42°C for 48 h in a microaerobic atmosphere using Campygen (CN025A, Oxoid, Basingstoke, UK). To enumerate *C. jejuni* by culture, 0.1 ml of each enrichment broth sample was streaked onto Campylobacter Selective Agars (modified CCDA, CM 739, Oxoid) and incubated microaerobically at 42°C for 48 h. Grown colonies demonstrating the characteristics of *Campylobacter* spp. were picked, purified and spesified further by Gram stain, motility test, growth in media containing 3.5 % NaCI, and 25 °C, 35-37°C and 42°C, oxidase reaction, hippurate hydrolysis, sensitivity to nalidixic acid (CT0031C, Oxoid) and cephalothin (CT0010B, Oxoid). The evaluation of MPN table was done according to De man (1983).

RESULTS

C. jejuni was not detected in the samples of Turkish fermented sucuk (n: 30), Turkish pastırma (n: 30), and raw beef meat samples purchased from the retail markets for use in manufacturing.

The initial numbers of *C. jejuni* were 10^2, 10^4, and 10^6 cfu/ml in the experimental Turkish fermented sucuk groups of I, II and III, respectively at pH 5.72 on the production day. The numbers of *C. jejuni* were found to be $<10^2$, 10^2, and 10^4 cfu/g in the experimental Turkish fermented sucuk groups of I, II, and III, respectively, indicating a two log reduction at pH 5.47 on day one. At the end of second day, pH was 5.32, and the numbers of *C. jejuni* were below the detection level of 10^2 cfu/g in all three experimental Turkish fermented sucuk groups and *C. jejuni* was not also detected by Most Probable Number (MPN) technique (<0.30MPN/g) in the same samples.

In the experimental Turkish pastırma samples, the number of *C. jejuni* was below the detection level of 10^2 cfu/g in group I while one log of reduction was observed in the groups of II and III at pH 5.58 on day one. These one log reductions continued in the groups of II and III untill the end of day three at pH 5.32. *C. jejuni* numbers were below the detection level of 10^2 cfu/g at the end of fourth day at pH 5.19 in the samples of all three experimental Turkish pastırma groups, and also *C. jejuni* was not detected by Most Probable Number (MPN) technique (<0.30MPN/g) in the same samples. The result of *C. jejuni* numbers in the experimental traditional Turkish fermented sucuk and Turkish pastırma samples are summarized in Table 1.

DISCUSSION

Red meat consumption is considered as an identified but minor risk factor for exposure to *Campylobacter* spp. As a medium for microbial growth, meat is suitable because of the high water and protein content. Not much information has been informed about the behaviour of *Campylobacter* spp. on beef or lamb meat during processing. Some information is available on retail fresh or frozen raw red and minced meat but not much about other red meat products. On meat cuts or minced meat, important factors for the behaviour of *C. jejuni* are temperature, medium, microaerophilic atmosphere, and pH. *Campylobacter* spp. usually survives poorly at atmospheric oxygen concentrations. Although *C.*

jejuni may not multiply readily in or on meat (Turnbull and Rose 1982), *Campylobacter* spp. can survive and even grow when initially packed under normal atmospheric conditions, as the metabolic activity of the food, for instance, raw meat may create a different gaseous environment (ICMSF 1996). Several researchers have reported that retail minced meat and sausages were contaminated with campylobacters (Bolton et al 1985, Turnbull and Rose 1982).

Table 1. The numbers of *C. jejuni* in the experimentally made traditional Turkish fermented sucuk and Turkish pastırma.

Days of production	Turkish fermented sucuk Level of inoculation (cfu/ml)				Turkish pastırma Level of inoculation (cfu/ml)			
	G1	G2	G3	pH*	G1	G2	G3	pH*
	10^2	10^4	10^6	5.72	10^2	10^4	10^6	5.75
1	$<10^2$	10^2	10^4	5.47	$<10^2$	10^3	10^5	5.58
2	$<10^2$	$<10^2$	$<10^2$	5.32	$<10^2$	10^2	10^3	5.42
3	$<10^2$	$<10^2$	$<10^2$	5.24	$<10^2$	$<10^2$	10^2	5.32
4	$<10^2$	$<10^2$	$<10^2$	5.03	$<10^2$	$<10^2$	$<10^2$	5.19
5	$<10^2$	$<10^2$	$<10^2$	5.00	$<10^2$	$<10^2$	$<10^2$	5.10
7	$<10^2$	$<10^2$	$<10^2$	5.02	$<10^2$	$<10^2$	$<10^2$	5.08
10	$<10^2$	$<10^2$	$<10^2$	5.02	$<10^2$	$<10^2$	$<10^2$	5.07
15	$<10^2$	$<10^2$	$<10^2$	5.03	$<10^2$	$<10^2$	$<10^2$	5.08

G1: Group I (inoculated with 10^2 cfu/ml *C. jejuni*); G2: Group I (inoculated with 10^4 cfu/ml *C. jejuni*)
G3: Group I (inoculated with 10^6 cfu/ml *C. jejuni*); *Initial pH

In this study, a very particular meat products of traditional Turkish fermented sucuk and Turkish pastırma were inoculated experimentally with *C. jejuni* at different levels of 10^2, 10^4 and 10^6 cfu/g to cover a broad range of numbers. Even though *C. jejuni* contamination of raw meat is generally considered low (Pezzotti et al 2003, Whyte et al 2004, Wong et al 2007) cross-contaminations occur as a risk in terms of uncooked meat products. During making Turkish fermented sucuk and Turkish pastırma, meat is neither cooked nor refrigerated, these products must be safe to consume yet. *C. jejuni* has been isolated with particular frequency in food products including ground meat and sausage (Baffone et al 1995, Bolton et al 1985). In minced meat, *C. jejuni* survived better and viable counts were relatively unchanged in minced beef at refrigerator temperatures and 22°C, but showed a decrease in corresponding samples of sausage mixture (Barrell 1984). However when it was stored at 20^0C, *C. jejuni* also decreased rapidly and could not be isolated after 3 days (Svedhem 1981). In the Chinese-style sausages, no viable *C. jejuni* was detected in either vacuum-packed or non-vacuum-packed sausage after 7 days of storage at 25°C (Yeh 1994). In the study of Bostan et al (2001), *C. jejuni* numbers on the 3rd day of fermentation was <10 cfu/g in the experimentally made Turkish fermented sucuk which were experimentally inoculated with *C. jejuni* at the numbers of 10^5 cfu/g. They, also reported that *C. jejuni* was not detected in their Turkish fermented sucuk samples on the 4th day of fermentation. On the other hand, in our study, *C. jejuni* was inactivated on the 2nd day of fermentation. While inactivation period was shorter in our study, inactivation pH level (pH 5.32) of this study was in paralell with those of Bostan et al (2001).

There are several factors that might effect the survival of *C. jejuni* in traditional Turkish fermented sucuk. The spices, garlic and nitrite-salt combination in the sucuk and çemen mixture may have inhibited *C. jejuni*. Thermophilic *Campylobacter* spp. are very sensitive to NaCl. The maximum NaCl concentration in which *Campylobacter* spp. which can multiply is 1.5% (Anon 1998) while optimum NaCl concentration for them is 0.5% (Doyle and Roman 1982, Hanninen 1981). Reduction due to organic and inorganic acids such as lactic acid occuring during the fermantation process and decreasing water activity are among other essential factors for lowering *C. jejuni* numbers, but it has been demonstrated that a strong bactericidal effect of organic acids on campylobacters occured much more between the pH values of 4.0 and 4.5 while bactericidal activity was low at values of pH 5.0 and above (Chaveerach et al 2002). In week acid conditions (pH between 5.0 and 6.0) *Campylobacter* spp. survived well and only minor decreases in culturability were found (Alter et al 2006, Waterman and Small 1988). Competitive flora such as *lactobacilli* may not have showed an antogonistic effect on the survival of *C. jejuni* as Koidis and Doyle (1983), and Moore and Madden (2001) reported that *Campylobacter* spp. behave differently than other food

pathogens in the presence of competitive flora. On the contrary, *Campylobacter* spp. are very sensitive to drying, particularly at ambient temperatures (Fernandez 1985, Lake et al 2007). In the study of Alter et al (2006), in paralel to reduction in water activity after 24-48 h ripening, detection of *C. jejuni* was only possible after enrichment in the German style fermented turkey sausages. Therefore, *C. jejuni* is likely to be inhibited at the temperatures of 20^0C and during drying at room temperature in our Turkish fermented sucuk and Turkish pastırma. There are other reports with either similiar or different results on inactivation periods and bacteria levels (Yeh and Chou 1994, Warburton, 1987). The varriations in inactivation periods may be due to different fermentation processes, ingredients and variations in the parameters of these process.

In conclusion, Bacterial carcass meat inspection is an important indicator for meat inspection. It must be done for public health because Turkish pastırma and Turkish style fermented sausage (sucuk) are raw meat products. Considering the result of this study and previous a few relevant studies, the risk of *C. jejuni* infection through consuming of traditional Turkish fermented sucuk and Turkish pastırma seems to be very low unless croos contamination occurs. However, due to very low infectious dose of *Campylobacter* spp. consuming unripened Tukish fermented sucuk and pastırma at the early days of fermentation (1-7 days) should be avoided without cooking.

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