

# Behavior of *Brucella abortus* and *Brucella melitensis* in Raw Meatball (Cig Kofte)

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## Abstract

In the present study, we aimed to determine the survival and reproducibility of the said pathogenic microorganisms during the storage period (24 h) of raw meatball samples contaminated with reference strains of *Brucella abortus* and *Brucella melitensis* at levels of  $10^4$  and  $10^6$  cfu/g. *Brucella abortus* (NCTC 11363) and *Brucella melitensis* (NCTC 10094) strains were incubated in sterile full-cream milk with 12 % fat at 37°C for 48 hours under a 6% CO<sub>2</sub> aerobic atmosphere. Subsequent to the incubation, culture counts were performed on Brucella Agar Base using tryptose soy broth. As a result during our analysis at the 12th hour of the storage, it was noted that while pH remained 5.2, aw 0.96 and moisture %52.16, population of *B. abortus* in-group A2 rose from  $10^4$  cfu/gr to  $10^5$  cfu/gr ( $P<0.05$ ). At hour 24, pH was found 5.1, aw 0.92 and moisture % 49.07 whereas population of *B. abortus* decreased to  $10^5$  cfu/gr in-group A1 and to  $10^4$  cfu/gr in-group A2 ( $P<0.05$ ). In-group B1, there was no change in the number of *B. melitensis*. It was also observed at hour 24 population of *B. melitensis* in-group B2 increased to  $10^5$  cfu/gr ( $P<0.05$ ).

**Keywords:** *Brucella abortus*, *Brucella melitensis*, Meat ball, Cig kofte, Traditional meat product

## 1. Introduction

Çiğ köfte (raw meatball) is a traditional meat dish/delicacy that is usually made and served as an appetizer in Turkey, particularly in Southeastern Anatolia, on special occasions and at communal events (Yıldırım *et al*, 2005). Since no standards exist for the preparation of çiğ köfte, the ingredients and amount of content vary depending on preference. It is a meat product that doesn't undergo any thermal treatment and is consumed raw. It is produced by kneading finely ground lean mutton or beef, with a mixture of bulgur (fine wheat groats), onion, garlic, paste, parsley, and spices (powdered isot, red pepper, black pepper, cinnamon, clove, cumin, mint) with water or ice (Uzunlu & Yıldırım, 2003; Yıldırım *et al*, 2005).

Studies conducted in Turkey on raw meatballs (Sagun *et al.*, 1997a; Kuplulu & Sarımehtmetoglu, 2003; Cetin *et al.*, 2008), have revealed that microbiological quality of the food appears hardly acceptable for human consumption and that it poses a significant threat to public health.

Brucellosis is a zoonotic infection, which has spread across many countries and remains endemic in developed ones, too (WHO, 2006). It is reported by the World Health Organisation (WHO) that an estimated 500,000 new human cases occur annually worldwide (Pappas *et al.*, 2006). Brucellosis is endemic almost in all regions of the world, including the Mediterranean countries such as Spain, Portugal, Southern France, Italy, Greece, Turkey; North Africa, the Near East, India, Mexico, and Central and South America (Yüce & Çavuş, 2006). For humans, the contraction of the disease arises from intake of raw or unpasteurized infected milk or dairy products. However inhalation of contaminated dust, and contact with infected carcasses, and uterine contents and discharges (Chahota *et al.*, 2003), and via consumption of meat and meat products (Robinson *et al.*, 2000).

In the present study, we aimed to determine the survival and reproducibility of the said pathogenic microorganisms during the storage period (24 h) of raw meatball samples contaminated with reference strains of *Brucella abortus* (NCTC 11363) and *Brucella melitensis* (NCTC 10094) at levels of  $10^4$  and  $10^6$  CFU/g, and to discover whether they pose a threat to public health or not.

## 2. Material and Methods

### 2.1 Preparation of raw meatballs

Raw Meatballs were made by mixing and kneading ground beef and bulghur, each ingredient in equal amounts of 2 kgs, powdered cumin, red pepper, black pepper, garlic, onion, parsley, tomato paste, and salt, based on the method as prescribed by Durmaz *et al.* (2007). The mixture was hand shaped into small balls each weighting approximately 25 g and stored at 4 °C for 24 hours. All çiğ köfte samples were analyzed in duplicate after storage for 0, 6, 12 and 24 h.

### 2.2 Microbiological analysis

#### 2.2.1 Preparation and Inoculation of Test Strains

*Brucella abortus* (NCTC 11363) and *Brucella melitensis* (NCTC 10094) strains were obtained from Refik Saydam National Public Health Agency and incubated in sterile full-cream milk with 12 % fat at 37°C for 48 hours under a 6% CO<sub>2</sub> aerobic atmosphere. Subsequent to the incubation, culture counts were performed on Farrell's Agar plates (Oxoid CM 169; Brucella Selective Supplement Oxoid SR 83) using tryptose soy broth as diluent in the range up to  $10^{-9}$ . The amount derived from the initial solutions inoculated showed that it could contaminate 1 g of raw meatball at levels of  $10^4$  cfu/g and  $10^6$  cfu/g. After the samples were inspected for any presence of *Brucella abortus* and *Brucella melitensis*, they were contaminated with strains of *B. abortus* and *B. melitensis* at levels of  $10^4$  cfu/g and  $10^6$  cfu/g (Estrada *et al.*, 2005). The prepared samples (A1:  $10^6$  cfu/g *B. abortus*; A2:  $10^4$  cfu/g *B. abortus*; B1:  $10^6$  cfu/g *B. melitensis*; B2:  $10^4$  cfu/g *B. melitensis*) were then stored at +4°C for 24 hours.

#### 2.2.2 Sampling and Preparation of Dilutions

Throughout the storage period, samples were taken from all raw meatballs at hours 0, 6, 12, and 24. 10 g specimens were weighed out into sterile Stomacher bags, and 90 ml of sterile peptone saline (% 0,85 NaCl + % 0,1 peptone) was added to each container. The samples were immediately homogenized in the stomacher (Interscience, UK) for 2 minutes and were diluted ten-fold. Following the homogenization, serial decimal dilutions were performed until reaching  $10^{-7}$  (Pichhardt, 1993).

#### 2.2.3 Enumeration of Total Mesophilic Aerobic Bacteria and Lactic Acid Bacteria

The dilutions prepared were planted in Plate Count Agar (PCA, Oxoid, CM325 – at 32°C for 48 h) to count the population of mesophilic aerobic bacteria, and in Man Rogosa Sharpe Agar (MRS, Oxoid, CM 361 – pH:5.7- at 35°C for 48 h, anaerobic) to count lactic acid bacteria (Pichhardt, 1993).

#### 2.2.4 Enumeration of *Brucella abortus* ve *Brucella melitensis*

25 g samples were weighed out from each raw meatball specimen under aseptic conditions and placed into sterile stomacher bags. 225 ml of BPW (Buffered Peptone Water, Oxoid CM0509) was added and homogenized. After being blended in the stomacher, the decimal dilutions obtained from homogenized samples were diluted ten-fold in BPW, being prepared until reaching  $10^{-9}$ . The samples were plated onto Farrell's agar (Oxoid CM 169; Brucella Selective Supplement Oxoid SR 83) by means of spread plate technique. The petri dishes planted were subjected to incubation at 37°C for 48 hours under a % 6 CO<sub>2</sub> environment. At the end of the incubation,

colonies with typical *Brucella* were enumerated and justified through agglutination tests (*B. abortus* antisera Difco 2871-47-7, *B. melitensis* antisera Difco 2889-47-7) (Estrada *et al.*, 2005).

### 2.3 Chemical analysis

pH values of the sampled raw meatballs were measured by means of pH metre (InoLab pH 720 model, Germany), based on method as prescribed by Troller and Scott (1992). Determination of moisture was achieved by AOAC (1990), and water activity (aw) values were analyzed according to the method by Rodel *et al.* (1975).

### 2.4 Statistics analysis

The data obtained from two replications were analysed by ANOVA using the SPSS statistical package program and differences among the means were compared using Duncan's Multiple Range test.

## 3. Result

Survival and reproducibility of the pathogenic microorganisms in raw meatball samples (A1:  $10^6$  cfu/g *B. abortus*, A2:  $10^4$  cfu/g *B. abortus*, B1:  $10^6$  cfu/g *B. melitensis*, B2:  $10^4$  cfu/g *B. melitensis*) contaminated with reference strains of *Brucella abortus* (NCTC 11363) and *Brucella melitensis* (NCTC 10094) at levels of  $10^4$  and  $10^6$  CFU/g can be seen in Table 1. During our analysis at the 6th hour of the storage, no change was recorded in the values of pH 5.3, aw 0.98 and moisture 64.10% as well as in numbers of *B. abortus* and *B. Melitensis* in all four groups. However, at the 12th hour of the storage, it was noted that while pH remained 5.2, aw 0.96 and moisture 52.16%, population of *B. abortus* in-group A2 rose from  $10^4$  cfu/gr to  $10^5$  cfu/gr ( $P<0,05$ ). At hour 24, pH was found 5.1, aw 0.92 and moisture 49.07% whereas population of *B. abortus* decreased to  $10^5$  cfu/gr in group A1 and to  $10^4$  cfu/gr in group A2. In group B1, there was no change in the number of *B. melitensis*. It was also observed at hour 24 that although values for pH (5.1), aw (0.92) and moisture (%49.07) dropped, population of *B. melitensis* in group B2 increased to  $10^5$  cfu/gr ( $P<0,05$ ).

## 4. Discussion

In the present study, we aimed to determine the survival and reproducibility of the said pathogenic microorganisms during the storage period (24 h) of çiğ köfte samples contaminated with reference strains of *Brucella abortus* (NCTC 11363) and *Brucella melitensis* (NCTC 10094) at levels of  $10^4$  and  $10^6$  CFU/g, and to discover whether they pose a threat to public health or not.

Studies carried out have showed that 54.7% of total food poisoning cases are caused by the consumption of meat and meat products (Farber *et al.*, 1989). A major epidemiological factor in contracting the disease lies in the fact that in certain societies people are in the habit of consuming raw meat, eg raw liver or other offal with spices. (Syrjamaki *et al.*, 1984). Following slaughter, micro-organisms, which cause foodborne infection and intoxication in humans, are transmitted to the end product as a result of cross-contamination during carcass boning/cutting, processing, packing, and storage, thereby posing a threat to consumer health. Researchers have reported that infected lymph nodes are particularly noted for their being the most likely source for endogenous contamination of ground meat (Erol, 1999a). Due to its texture and processing techniques, ground meat turns out to be the leading meat product suitable for microbial contamination (Sinell, 1992).

Under the present study, in our analysis on the sample raw meatballs at the 6th hour of the storage, the original values of pH 5.3, aw 0.98 and moisture % 64,10 showed no change. Also, numbers of *B. abortus* and *B. Melitensis* didn't alter in any of the four groups: A1, A2, B1 and B2. At hour 12 of the storage, however, it was noted that while pH remained 5.2, aw 0.96 and moisture 52.16%, population of *B. abortus* in group A2 rose from  $10^4$  cfu/gr to  $10^5$  cfu/gr. Our measurement at the 24th hour revealed pH 5.1, aw 0.92 and moisture % 49.07, whereas population of *B. abortus* decreased to  $10^5$  cfu/gr in group A1 and to  $10^4$  cfu/gr in that of A2. In group B1, no change was noted in the number of *B. melitensis*. It was also observed that although values for pH (5.1), aw (0.92) and moisture (49.07%) dropped, population of *B. melitensis* in group B2 increased to  $10^5$  cfu/gr.

Optimum pH value for the reproduction of *Brucella* spp. is 6,6-7,4, with maximum 8.7 and minimum 5.8 (Frobisher, 1968). However, various studies have yielded results which exhibit that *Brucella* spp can somehow survive even at lower pH values in different foods. Robinson *et al.* (2000), reported that *Brucella abortus* managed to survive for 34 days in the milk set at pH 5.0-5.8 by means of lactic acid, and for 2 days at pH 3.9. In the study carried out by Estrada *et al.* (2005), sterile skim milk was inoculated with *Brucella abortus* at  $10^5$  cfu/g with a yoghurt starter culture of lactic acid bacteria, and was incubated at +4°C. Their results demonstrated that after 10 days of storage at 4 degrees C, *B. abortus* was recovered in fermented milk at a level of  $10^5$  cfu/g, despite the low pH value below 4.0. Ozturk and Nazli (1996), have reported that they experimentally contaminated sheep cheese and cow's cheese stuffed into sheepskin bags with *Brucella melitensis* at  $10^9$  cfu/g, and that the pathogen decreased to  $10^2$  cfu/g in both cheese varieties on the 21st day of the maturation. However,

*Brucella melitensis* couldn't be isolated at pH 5.0 in either samples on the 30th day of the maturation. Researchers have also documented that following contamination, *Brucella* spp. managed to survive for 14 days in chilled meat (Taşkın, 2007), and for several years in frozen tissues and organs (Altekruse *et al.*, 1998).

This experimental study demonstrated that while levels of reference strains *B. abortus* and *B. melitensis* in raw meatball samples stored for 24 hours at +4 °C did not change significantly, the total population of mesophilic aerobic bacteria ( $10^7$  cfu/g) increased (Table 1). When the literature was screened, no similar experimental study related to meat and meat products occurred. As to experimental studies on milk and dairy products, while Ozturk and Nazlı (1996) observed that *brucellae* in cheese were inhibited at pH 5.0, Robinson *et al.* (2000) and Estrada *et al.* (2005), found that they were not inhibited even at lower pH levels (3.9 and 4.0). In the present study, at 24-hour storage period, pH ranged between 5.3 - 5.1, and *B. abortus* and *B. melitensis* were not inhibited at pH 5.1. The fact that *B. abortus* and *B. melitensis* survive for different time periods or is inhibited at different pH levels may depend on products' composition; amount of moisture, protein and fat content; their texture; and storage period and conditions.

Çiğ köfte is a traditional and popular meat dish/delicacy throughout Turkey; however, since it is consumed raw, it gives rise to the spread of various pathogenic bacteria as well as *Brucella* outbreaks and poses a major threat to public health. Indeed, it has been documented by different investigators in Turkey that sanitary quality of ground meat, which is the basic ingredient for making çiğ köfte (Tekinşen *et al.*, 1980; Yetim, 1985; Akın & Kaya, 1988; Sancak *et al.*, 1993; Ciftcioglu & Ugur, 1992; Guven *et al.*, 1997; Erol, 1999b; Sireli & Erol, 1999), as well as the spices used (Sagun *et al.*, 1997b; Erol *et al.*, 1999; Vural, 2004) is hazardous for public health.

Antimicrobial effect of spices added to çiğ köfte is not powerful enough to eliminate the risk of pathogenic microorganisms. Because the antibacterial effect of spices such as isot and black pepper used in the preparation of çiğ köfte is limited, population of microorganisms doesn't undergo any significant change under different temperatures and periods (Uzunlu & Yıldırım, 2003). Experimental studies have demonstrated that many pathogenic bacteria can sustain their survival and are not inhibited in çiğ köfte environments (Sagun *et al.*, 2003; Uzunlu & Yıldırım, 2003; Sireli *et al.*, 2008). Therefore, the folk belief that added spices will thoroughly kill the pathogenic microorganisms in raw meatballs remains only a myth.

Brucellosis remains a major public health issue in Turkey. Varying rates of *Brucella* prevalence among humans have been noted by researchers in our country (Durmaz *et al.*, 1997; Altındış, 2001; Sumer *et al.*, 2003; Atmaca *et al.*, 2004; Cetinkaya *et al.*, 2005; Demirturk *et al.*, 2008). In Turkey, brucellosis is a particularly common problem in dairy cattle (Gokcen and Eskiizmirliler, 1998; Iyisan *et al.*, 2000; Solmaz *et al.*, 2002; Ceylan *et al.*, 2003) and in sheep (Gokcen & Eskiizmirliler, 1998; Muz *et al.*, 1999; Iyisan *et al.*, 2000; Ongor *et al.*, 2001; Ceylan *et al.*, 2003). It is unfortunate that sale and slaughtering of animals suffering from *brucella* still continues in abattoirs due to negligence and lack of proper inspection at livestock markets and slaughterhouses. Meat and ground meat obtained from such animals may end up at points of sale, posing a risk for public health. Fearing possible health risks, some consumers have, in recent years, turned to çiğ köfte completely made with bulgur as a replacement for ground meat. We suggest that a more pro-active and pre-emptive policy should be adopted to eradicate *brucella* infections, which is especially widespread in dairy cattle and sheep in Turkey; that procedures and practices like HACCP and GMP should be rigorously implemented during the production phase of meat and meat products by following the "from farm to table" continuum; that all the community working in the field of animal husbandry should be trained; that alternative methods should be taken up for making this traditional meat product, such as preparing çiğ köfte without adding any ground meat, or exposing it to thermal treatment in a way that will not spoil its characteristics; that different methods, such as food irradiation techniques should be developed to enhance the microbiological quality of çiğ köfte; and that training courses and seminars should be organized for çiğ köfte producers so that awareness could be raised among them.

## References

- Akın, A., & Kaya, B. (1988). Ankara'da Satılan Etlerin Mikrobiyolojik Kalite Kontrolü, 1-Kıymaların Mikrobiyolojik Kalite Kontrolü. *Doğa Tıp ve Ecz. Derg.* 12, 183-189.
- Altekruse, S. F., Timbo, B. B., & Mawbroyi, J. C. (1998). Cheese-associated outbreaks of human illness in the United States. *Journal of Food Protection*, 61(10), 1405-1407.
- Altındış, M. (2001). Afyon Bölgesi Besicilerinde, Kasaplarda, Süt Ürünleri Toplayıcısı, imalathanelerinde Çalışanlarda Bruselloz Seropozitifliği. *İnfeksiyon Dergisi*, 15, 11-15.
- AOAC. (1990). Method 926.08, 925.09. Association of Official Analytical Chemists. Official Methods of Analysis. (15th ed). AOAC, Arlington: USA.

- Arslan, A., Guven, A., Saltan, S., & Patir, B. (1992). Microbiologic quality of raw meat balls in Elazig. Firat University, *Health Science Journal*, 6, 13-18.
- Atmaca, S., Özekinci, T., Akpolat, N., Elçi, S., Suay, A., & Asikan, E. (2004). Brucellosis Seroprevalance in Southeast Turkey (Diyarbakır). *Turkish Journal of Medical Sciences*, 34, 251-255.
- Cetin, O., Bingol, E. B., & Akkaya, H. (2008). The Microbiological, Serological and Parasitological Quality of Cig Kofte (Raw Meatball) and Its Lettuce Marketed in Istanbul Polish. *Journal of Environmental Studies*, 17, 701-706.
- Çetinkaya, Z., Aktepe, O. C., Çiftci, I. H., & Demirel, R. (2005). Seroprevalence of Human Brucellosis in a Rural Area of Western Anatolis, Turkey. *Journal of Health Population and Nutrition*, 23, 137-141.
- Ceylan, E., Irmak, H., Buzğan, T., Karahocagil, M. K., Evirgen, Ö., Sakarya, N., Akdeniz, H., & Demiröz, A. P. (2003). Van İline Bağlı Bazı Köylerde İn san ve Hayvan Populasyonunda Bruselloz Seroprevalansı. *Van Tıp Dergisi*, 10, 1-5.
- Chahota, R., Sharma, M., Katoch, R. C., Verma, S., Singh, M. M., Kapoor, V., & Asrani, R. K. (2003). Brucellosis outbreak in an organized dairy farm involving cows and in contact human beings, in Himachal Pradesh, India. *Veterinarski Arhiv*, 73, 95-102.
- Çiftçiöğlu, G., & Uğur, M. (1992). Kıyma, sucuk ve tavuk etlerinde *Listeria monocytogenes* kontaminasyonu, *İstanbul Üniversitesi Veteriner Fakültesi Dergisi*, 18, 33-44.
- Demirturk, N. T., Demirdal, N., Erben, S., Demir, Z., & Asci, T. P. (2008). Brucellosis: A retrospective evaluation of cases and review of Brucellosis treatment. *Tropical Doctor*, 38, 59-62. <http://dx.doi.org/10.1258/td.2006.006266>.
- Durmaz, H., Sagun, E., Sancak, H., & Sagdic, O. (2007). The fate of two *Listeria monocytogenes* serotypes in “cig kofte” at different storage temperatures. *Meat Science*, 76, 123-127. <http://dx.doi.org/10.1016/j.meatsci.2006.10.020>.
- Durmaz, R., Durmaz, B., Rafiq, M., & Sönmez, E. (1997). Seropositivity of Brucellosis in Malatya, Turkey. *Turkish Journal of Medical Sciences*, 27, 125-128.
- Erol, İ. (1999a). Hayvansal gıdalardan kaynaklanan Salmonella Enfeksiyonları. *İnfeksiyon Dergisi*, 13, 123-127.
- Erol, İ. (1999b). Ankara’da tüketime sunulan kıymalarda Salmonellaların varlığı ve serotip dağılımı. *Turkish Journal of Veterinary and Animal Sciences*, 23, 321-325.
- Erol, İ., Küplülü, Ö., & Karagöz, S. (1999). Ankara’da tüketime sunulan bazı baharatın mikrobiyolojik kalitesi. *Ankara Veteriner Fakültesi Dergisi*, 46, 115-125.
- Estrada, A. Z., De La Garza, L. M., Mendoza, M. S., Lopez, E. M. S., Kerstupp, S. F., & Merino, A. L. (2005). Survival of *Brucella abortus* in milk fermented with a yoghurt starter culture. *Revista Latinoamericana de Microbiologia*, 47, 88-91.
- Farber, J. M., Sanders, G. W., & Johnston, M. A. (1989). A survey of various foods for the presence of *Listeria* species. *Journal of Food Protection*, 52, 456-458.
- Frobisher, M. (1968). *Fundamentals of Microbiology* (8th ed). Press of W.B. Saunders company, Philadelphia, London: Toronto. 740.
- Gökçen, S., & Eskiismirliler, S. (1998). Bornova Veteriner Kontrol Ve Araştırma Enstitüsü’ne 1988-1997 Yılları Arasında Ege Bölgesi İllerinden Gönderilen Sığır, Koyun Ve Keçi Kan Serumlarında *Brucella* Pozitiflik Oranı. *Bornova Veteriner Kontrol ve Araştırma Enstitüsü Müdürlüğü Dergisi*, 23, 1-10.
- Güven, A., Gülmez, M., & Kamber, U. (1997). Kars ilinde tüketime sunulan kıymalarda bazı patojen mikroorganizmaların araştırılması ve kıymaların mikrobiyolojik kalitesinin belirlenmesi. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 3, 57-65.
- İyisan, A. S., Akmaz, O., Duzgun, S. G., Ersoy, Y., Eskiismirliler, S., & Guler, L. (2000). Sero-epidemiology of Brucellosis on cattle and sheep in Turkey. *Journal of Pendik Veterinary Microbiology*, 31, 21-75.
- Küplülü, Ö., & Sarımehtemoğlu, B. (2003). The Microbiological Quality of Çiğ Köfte Sold in Ankara. *Turkish Journal of Veterinary and Science*, 27, 325-329.
- Muz, A., Ertaş, H. B., Öngör, H., Gülcü, H. B., Özer, H., Eröksüz, H., Dabak, M., Başbuğ, O., & Kalender H. (1999). Elazığ ve Çevresinde Koyun ve Keçilerde Abortus Olgularının Bakteriyolojik, Serolojik ve Patolojik Olarak İncelenmesi. *Journal of Veterinary and Animal Sciences*, 23, 177-188.

- Nielsen, K. H., Kelly, L., Gall, D., Balsevicius, S., Basse, J., Nicoletti, P., & Kelly, W. (1996). Comparison of enzyme immunoassays for the diagnosis of bovine Brucellosis. *Preventive Veterinary Medicine*, 26, 17-32.
- Ocal, H. M. (1997). *Ozellikleri ve Guzellikleriyle Cig Koftemiz*. Ozlem Kitabevi. Sanliurfa. Turkey.
- Öngör, H., Muz, A., & Çetinkaya, B. (2001). Atık yapmış koyunlarda Brucellozis'in teşhisinde ELISA ile diğer serolojik testlerin karşılaştırılması. *Turkish Journal of Veterinary Animal Sciences*, 25, 21-26.
- Öztürk, G. Y. & Nazlı, B. (1996). Deneyisel olarak enfekte edilen sütte yapılan tulum peynirlerinde *Brucella melitensis*'in mevcudiyeti üzerine araştırmalar. *Pendik Veteriner Mikrobiyolojisi Dergisi*, 27, 123-142.
- Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., & Tsianos, E. V. (2006). The new global mab of human Brucellosis. *Lancet Infectious Diseases*, 6, 91-99. [http://dx.doi.org/10.1016/S1473-3099\(06\)70382-6](http://dx.doi.org/10.1016/S1473-3099(06)70382-6)
- Pichhardt, K. (1993). *Lebensmittel-Mikrobiologie*. Springer Verlag: Berlin.
- Robinson, R. K., Batt, C. A., & Patel, P. D. (2000). *Encyclopedia of food Microbiology*. In Brucella. (ed) Academic pres., London, 1, 319-327.
- Rödel, W., Panert, H., & Leistner, L. (1975). Verbessertes aw-Wert-Messer zur Bestimmung der Wasseraktivität von Fleisch und Fleisch Waren. *Fleischwirtschaft*, 4, 557-558.
- Sagun, E., Alişarlı, M., & Durmaz, H. (2003). Farklı Sıcaklıklarda Muhafazanın Çiğ Köftede *Staphylococcus aureus*'un Gelişimi ve Enterotoksin üretimi üzerine Etkisi. *Turkish Journal of Veterinary Animal Sciences*, 27, 839-845.
- Sagun, E., Sancak, Y. C., Durmaz, H., & Akkaya, L. (1997a). A study on hygienic quality of raw meat balls consumed in Van. *Yuzuncu Yil University Veterinary Faculty Journal*, 3, 64-67.
- Sagun, E., Sancak, Y. C., Durmaz, H., & Ekici, K. (1997b). Van'da tüketime sunulan baharatların mikrobiyolojik kalitesi, *Yuzuncu Yil University Veterinary Faculty Journal*, 8, 1-5.
- Sancak, Y. C., Boynukara, B., & Ağaoğlu, S. (1993). Van'da Tüketime Sunulan Kıymaların Mikrobiyolojik Kalitesi. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi*, 4, 73-86.
- Sinell, H. J. (1992). Faktoren, die mikrobiellen Verderb bestimmen. In: Einführung in die Lebensmittelhygiene, Paul Prey Verlag, Hamburg: Berlin, 85-105.
- Şireli, U. T., & Erol, İ. (1999). Hazır kıymalarda Listeria türlerinin araştırılması. *Turkish Journal of Veterinary Animal Sciences*, 23, 373-380.
- Şireli, U. T., Göncüoğlu, M., & Pehlivanlar, S. (2008). Growth of *Listeria monocytogenes* in çiğ köfte (raw meat ball). *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 55, 83-87. [http://dx.doi.org/10.1501/Vetfak\\_0000000304](http://dx.doi.org/10.1501/Vetfak_0000000304).
- Solmaz, H., Tütüncü, M., Gülhan, T., Ekin, İ. H., & Talaş, İ. (2002). Van Yöresi Süt Sığırlarında Brucellozis'in İnsidensi Üzerine İnceleme. *Yuzuncu Yil University Veterinary Faculty Journal*, 13, 54-56.
- Sümer, H., Sümer, Z., Alim, A., Nur, N. & Özdemir, L. (2003). Seroprevalence of Brucella in an Elderly Population in Mid Anatolia, Turkey. *Journal of Health Population and Nutrition*, 21, 158-161.
- Syrjamäki, C., Migliazzo, A., Yarbough, J., & Le, M. (1984). Brucella abortus Endocarditis Following Ingestion of Cow's Blood. *Nebraska Medical Journal*, 69, 141-142.
- Taşkın, N. (2007). Ankara ilinde tüketime sunulan sokak sütlerinde ve beyaz peynirlerde Brucella varlığının ELISA yöntemi ile araştırılması ve Brucella bakterisinin kefirde yaşam süresinin tayini. Gazi Üniversitesi Fen Bilimleri Enstitüsü. Yüksek Lisans Tezi. Ankara.
- Tekinşen, O. C., Yurtyeri, A., & Mutluer, B. (1980). Ankara'da Satılan Hazır Kıymalarının Bakteriolojik Kalitesi. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 27, 45-63. [http://dx.doi.org/10.1501/Vetfak\\_0000000885](http://dx.doi.org/10.1501/Vetfak_0000000885).
- Troller, J. A., & Scott, V. N. (1992). Measurement of water activity (Aw) and acidity. In: Compendium of Methods for the Microbiological Examination of Foods (edited by C. Vanderzant and D.F. Splittstoesser), (3rd ed.). American Public Health Association. Washington, DC.
- Uzunlu, S., & Yıldırım, I. (2003). Microbiological quality of raw meat ball and investigation of its microbial variation at the different storage time and temperature. *Gıda*, 28, 553-558.
- Vural, A. (2004). Investigation on the microbiological quality of nonpacking some spices in Diyarbakır province. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 10, 13-18.

WHO. (2006). Brucellosis in humans and animals. WHO Press, World Health Organization. Geneva: Switzerland.

Yetim, H. (1985). Erzurum piyasasında tüketime sunulan sığır kıymalarının bazı saprofit ve bir kısım patojen bakteriler yönünden incelenmesi, Atatürk Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi. Erzurum.

Yıldırım, İ., Uzunlu, S., & Topuz, A. (2005). Effect of gamma irradiation on some principle microbiological and chemical quality parameters of raw Turkish meat ball. *Food Control*, 16, 363-367. <http://dx.doi.org/10.1016/j.foodcont.2004.04.001>.

Yüce, A., & Çavuş, S.A. (2006). Türkiye’de Bruselloz: Genel Bakış. *Klinik Dergisi*, 19, 87-97.

Table 1. Behavior of *Brucella abortus* and *Brucella melitensis* in Raw Meatball

| Groups | Holding time (h) | <i>Brucella</i> (cfu/g) | TAMB (cfu/g)      | LAB (cfu/g)       | pH                | a <sub>w</sub>    | Humidity (%)       |
|--------|------------------|-------------------------|-------------------|-------------------|-------------------|-------------------|--------------------|
| A1     | 0                | 6,20 <sup>b</sup>       | 4,00 <sup>d</sup> | 3,04 <sup>d</sup> | 5,30 <sup>a</sup> | 0,98 <sup>a</sup> | 64,23 <sup>a</sup> |
|        | 6                | 6,45 <sup>a</sup>       | 4,66 <sup>c</sup> | 4,08 <sup>c</sup> | 5,30 <sup>a</sup> | 0,97 <sup>a</sup> | 64,11 <sup>b</sup> |
|        | 12               | 6,08 <sup>b</sup>       | 5,82 <sup>b</sup> | 4,34 <sup>b</sup> | 5,20 <sup>b</sup> | 0,95 <sup>b</sup> | 52,16 <sup>c</sup> |
|        | 24               | 5,56 <sup>c</sup>       | 7,72 <sup>a</sup> | 4,41 <sup>a</sup> | 5,10 <sup>c</sup> | 0,91 <sup>c</sup> | 49,07 <sup>d</sup> |
| A2     | 0                | 4,70 <sup>b</sup>       | 4,32 <sup>d</sup> | 3,26 <sup>d</sup> | 5,30 <sup>a</sup> | 0,98 <sup>a</sup> | 64,23 <sup>a</sup> |
|        | 6                | 4,56 <sup>b</sup>       | 4,65 <sup>c</sup> | 4,36 <sup>c</sup> | 5,30 <sup>a</sup> | 0,97 <sup>a</sup> | 64,12 <sup>a</sup> |
|        | 12               | 5,08 <sup>a</sup>       | 5,81 <sup>b</sup> | 4,52 <sup>a</sup> | 5,20 <sup>b</sup> | 0,95 <sup>b</sup> | 52,16 <sup>b</sup> |
|        | 24               | 3,30 <sup>c</sup>       | 7,75 <sup>a</sup> | 4,40 <sup>b</sup> | 5,10 <sup>c</sup> | 0,91 <sup>c</sup> | 49,07 <sup>c</sup> |
| B1     | 0                | 5,60 <sup>c</sup>       | 4,34 <sup>d</sup> | 3,15 <sup>d</sup> | 5,30 <sup>a</sup> | 0,98 <sup>a</sup> | 64,23 <sup>a</sup> |
|        | 6                | 6,72 <sup>a</sup>       | 4,66 <sup>c</sup> | 4,38 <sup>c</sup> | 5,30 <sup>a</sup> | 0,97 <sup>a</sup> | 64,11 <sup>b</sup> |
|        | 12               | 6,20 <sup>b</sup>       | 5,88 <sup>b</sup> | 4,61 <sup>a</sup> | 5,20 <sup>b</sup> | 0,95 <sup>b</sup> | 52,16 <sup>c</sup> |
|        | 24               | 5,48 <sup>c</sup>       | 7,77 <sup>a</sup> | 4,45 <sup>b</sup> | 5,09 <sup>c</sup> | 0,91 <sup>c</sup> | 49,07 <sup>d</sup> |
| B2     | 0                | 4,30 <sup>c</sup>       | 4,51 <sup>d</sup> | 3,11 <sup>d</sup> | 5,30 <sup>a</sup> | 0,98 <sup>a</sup> | 64,23 <sup>a</sup> |
|        | 6                | 4,56 <sup>b</sup>       | 4,64 <sup>c</sup> | 4,04 <sup>c</sup> | 5,29 <sup>a</sup> | 0,97 <sup>a</sup> | 64,12 <sup>b</sup> |
|        | 12               | 4,11 <sup>d</sup>       | 5,86 <sup>b</sup> | 4,52 <sup>a</sup> | 5,20 <sup>b</sup> | 0,95 <sup>b</sup> | 52,15 <sup>c</sup> |
|        | 24               | 5,11 <sup>a</sup>       | 7,70 <sup>a</sup> | 4,38 <sup>b</sup> | 5,10 <sup>c</sup> | 0,91 <sup>c</sup> | 49,07 <sup>d</sup> |

A1: 10<sup>6</sup> cfu/gr *B. abortus*; A2: 10<sup>4</sup> cfu /gr *B. abortus*; B1: 10<sup>6</sup> cfu /gr *B. melitensis*; B2: 10<sup>4</sup> cfu /gr *B. melitensis*;

TAMB: Total Aerobic Mesophilic Bacteria; LAB: Lactic Acid Bacteria

a-d Means in a same column with different letters are significantly different (p<0,05).