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RESEARCH ARTICLE

ARAŞTIRMA MAKALESİ

Detection of prevalence and contamination level of *Brucella* spp. in local cheese produced in Afyonkarahisar, Turkey

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SUMMARY

In this study, it was aim to determine presence and contamination level *Brucella* spp. in 100 home made fresh white cheese (not brined and aged cheese) with 50 home made and 50 dairy product totaly 100 tulum cheese (aged skin bag cheese) which were produced locally in Afyonkarahisar and sold at the bazaar. During this study, 200 cheese samples were evaluated in December 2011-January-February, 2012. *Brucella* spp. from cheese samples were isolated and identified according to Farell (1974), to determine the contamination level of *Brucella* MPN technique was performed. *Brucella* spp. were isolated all sample 14.2%. from all of the cheese samples (200). Contamination level of *Brucella* spp. in most samples was detected as <3.6 MPN/g, in five samples 3.6 MPN/g, in three samples 6.1 MPN/g, in four samples 7.4 MPN/g and in two samples 3.0 MPN/g. *Brucella* spp. were detected in 6 samples (6%) of tulum cheese and 8 samples (8%) of fresh white cheese. From all of the samples totally 28 isolates were obtained and 18 (64.2%) of them were *Brucella abortus*, 10 (35.7%) of them were *Brucella melitensis*. All of the isolated strains showed resistance to amoxicilline/clavulanic acid, penicilline, tetracycline, colistin and danofloxacin.

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Afyonkarahisar'da Lokal Olarak Üretilen Bazı Peynirlerdeki *Brucella* Spp.'nin Varlığı ve Kontaminasyon Düzeyinin Belirlenmesi

ÖZET

Bu çalışmada, Aralık 2011-Ocak-Şubat 2012 döneminde, lokal olarak üretilen ve pazarda satışa sunulan 100 ev yapımı taze beyaz peynir (salamura ve olgunlaşma yapılmamış) ve 50'si ev yapımı, 50'si mandıra ürünü olmak üzere 100 tulum peyniri örneğinde *Brucella* spp.'nin varlığı ve kontaminasyon düzeyinin belirlenmesi amaçlanmıştır. *Brucella*'nın izolasyon ve identifikasyonu, Farell (1974)'e göre, kontaminasyon düzeyi En Muhtemel Sayı Tekniği'ne (EMS) göre yapıldı. Tüm örneklerden (200) %14.2 düzeyinde *Brucella* spp. izole edildi. Çoğu örneğin kontaminasyon düzeyi <3.6 MPN/g, 5 örneğin 3.6 MPN/g, 3 örneğin 6.1 MPN/g, 4 örneğin 7.4 MPN/g ve 2 örneğin 3.0 MPN/g olarak belirlendi. Tulum peyniri örneklerinin 6'sından, taze beyaz peynir örneklerinin 8'inden *Brucella* spp. saptandı. Tüm örneklerden 28 izolat elde edilirken, bunların 18'i (%64.2) *Brucella abortus*, 10'u (35.7) *Brucella melitensis* olarak identifiye edildi. Elde edilen izolatların tümü amoksisilin/klavulanik asit, penisilin, tetrasiklin, kolistin ve danofloksasin'e dirençli olduğu belirlendi.

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INTRODUCTION

Brucellosis is an infectious disease primarily of domestic and wild animals caused by coccobacilli of the genus *Brucella* and transmissible to human through direct contact with infected animals and consumption of infected animal products (Sauret et al., 2002). Particularly, milk and dairy products are a valuable source of nutrition, but may harbour the following pathogens or be exposed production to contamination from various animal, environmental or human sources. Pathogens of animal origins as *Brucella*, the most important source may be extracted in milk, but more commonly contamination occurs during milking from the skin (Sharp, 1987; Sauret and Vilissova, 2002).

Brucellae are Gram-negative, facultative intracellular bacteria that can infect many species of animals and man. Six species are recognized within the genus *Brucella*: *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Brucella ovis*, *Brucella canis*, and *Brucella neotomae* (Godfroid et al., 2005). This classification is mainly based on the difference in host preference and in pathogenicity. Worldwide, the main pathogenic species for domestic animals are *B. abortus*, responsible for bovine brucellosis; *B. melitensis*, the main etiologic agent of small ruminant brucellosis; and *B. suis* responsible for swine brucellosis. These three *Brucella* species may cause abortion in their hosts and because of the presence of brucellosis in a herd (or flock), a region or a country, international veterinary regulations impose restrictions on animal movements and trade, which result in economic losses (Anonymous 1997; Anonymous 2003). Infected cows, the udder and supramammary lymph node are the most common sites for localization (Corner et al., 1987; Fensterbank, 1987). The different isolation rates were reported on *Brucella* species connected with mastitis in various countries (Mdegela et al., 2004; O'Bleness & Van Vlevk, 1962; White et al., 1986).

Common routes of infection include inoculation through cuts and abrasions in the skin or via the conjunctival sac of the eyes, inhalation of infectious aerosols, and ingestion via the gastrointestinal tract. Susceptibility to infection depends upon various factors, including the nutritional and immune status of the host, the size and route of the inoculum, and the species of *Brucella* causing the infection. In general, *B. melitensis* and *B. suis* are more virulent for humans than are *B. abortus* and *B. canis*. (Ariza et al., 1993; Young 1995).

The epidemiology of human brucellosis, the commonest zoonotic infection worldwide, has drastically changed over the past decade because of various sanitary, socioeconomic, and political

reasons, together with the evolution of international travel. Several areas traditionally considered to be endemic—eg, France, Israel, and most of Latin America—have achieved control of the disease. On the other hand, new foci of human brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the near east (eg, Syria) is rapidly worsening. Furthermore, the disease is still present, in varying trends, both in European countries and in the USA. Awareness of this new global map of human brucellosis will allow for proper interventions from international public-health organisations (Pappas et al., 2006).

Areas currently considered to have high brucellosis prevalence areas are the Middle East, the Mediterranean Basin (Portugal, Spain, Italy, Greece, Turkey, Near East, North Africa), South and Central America, South-Eastern Europe, Asia, Africa, and the Caribbean. (Abbas&Agab 2002; Abou-Eisha 2000; Awad 1998; Cooper 1992; Refai 2002). Overall, consumption of milk or cheese products from Mexico implicated in 45% of cases reported from California from 1973 to 1992 (Chomel et al., 1994). Because the proportion of cases due to food borne transmission was higher in the latter half of this period, researchers assumed that currently 50% of cases are foodborne (Mead, 1999; Young, 2000, Young, 1991, Young, 1995).

Epidemiological studies conducted have reported raw milk and milk products as the cause of brucellosis. (Garin-Bastuji & Verger, 1994; Herr & Roux, 1981; Jawetz et al., 1987). The consumption of unpasteurized dairy products is one of the important sources of human brucellosis. Fresh white cheese which is produced locally and sometimes privately in country houses from raw goat and sheep milk is probably the most important way of transmission (Sabbaghian, 1975). Although the consumption of dairy products made from raw milk is assumed to be a higher health risk for the potential occurrence of human pathogens, (West, 2008; Claeys et al., 2012), raw milk cheeses are generally perceived by consumers as food products with premium quality and higher flavour intensity (Colonna et al., 2011; Muir et al., 1997). By contrast, pasteurised milk cheeses, which are generally produced by large-scale dairy industries with modern technologies under carefully controlled conditions, are claimed to have a less intense sensory profile (Beuvier et al., 1997; Colonna et al., 2011; Muir et al., 1997), due to killing of much of the indigenous microbiota of the raw milk and replacement with a few selected starter strains (Psoni et al., 2006).

We know that sheep, goats and cow were the primary domestic animals in Turkey. Small ruminants raw milk was used to make cheese, one of the primary ingredients in Afyonkarahisar. It was therefore hypothesized that milk and milk products were important sources of an infectious food-borne disease that was later known as the brucellosis due to *Brucella melitensis* and *Brucella abortus*.

Brucellosis is still an important infectious disease being widespread as endemic and sporadic cases in Turkey. High risk families should be educated about appropriate cooking of milk and milk products (Çelebi, 2007). Afyonkarahisar is located in the western region of Turkey and is becoming significant as a breeding area for sheep, dairy cows and water buffaloes owing to suitable conditions. The milk and milk products such as tulum cheese, white cheese, lor cheese and butter obtained from these animals are the sought-after Turkish products. Tulum cheese is one of the most popular semi-hard cheeses in Turkey, both of white cheese and tulum cheese is produced from raw sheep, goat or cow milk. An increase in the annual number of cases reported has been also observed in Turkey, exceeding 15,000 cases in 2004 (WHO, 2004). Brucellosis is particularly endemic in the poor eastern regions. Numerous reports have addressed the epidemiology of the disease in isolated areas from this region, (Aygen et al., 2002) although a coordinated national approach is still missing. The relation of poor socioeconomic status and brucellosis incidence, as outlined in the EU, probably applies here too: numerous studies focusing on seroprevalence, summarised by Cetinkaya and colleagues, (Çetinkaya et al., 2005) show that the proximity of a region either to Europe or to Ankara is accompanied by lower seroprevalence rates, possibly due to enhanced application of the Turkish Brucellosis Challenge Project that has been running for the past 20 years.

Antimicrobials are used in food animals to treat or prevent disease and also to promote growth (Merck, 1998). Generally, tetracyclines, penicillins, sulfonamides may be administered to treat (Friendship, 2000). Although lactating dairy cows receive few antimicrobials in feed, antimicrobials (penicillins, cephalosporins, erythromycin, and oxtetracyclines) are administered through intramammary infusion to treat mastitis, (Erskine, 2000; NASCD, 1999). Such drugs are often routinely administered to entire herds to prevent mastitis during nonlactating periods (Erskine, 2000). Several recent reviews survey antimicrobial resistance across many animal species (ACMS, 1999; WHO, 1998). In animals, antimicrobial resistance in zoonotic enteropathogens are of special concern to human

health because these bacteria are most likely to be transferred through the food chain to humans, or resistance genes in commensal bacteria may be transferred to the zoonotic enteropathogens (Salysers, 1995). There is considerable evidence that antimicrobial use in animals selects for resistance in commensals (Linton et al., 1975; Bager et al., 1997) and in zoonotic enteropathogens (Endtz et al., 1991; Low et al., 1997).

For this reason in this study it was aimed to detect prevalence and contamination level of *Brucella* spp. in local cheese and detected antibiotic susceptibility of isolates.

MATERIAL AND METHODS

Materials

Two hundred samples of milk product (100 samples of tulum cheese, 100 of fresh white cheese) were purchased at two different bazaars in the Afyonkarahisar December 2011 - January-February, 2012. Samples were analyzed immediately after they were brought to the laboratory in the carrying bowls under aseptic conditions kept in the cold chain.

Methods

Isolation and identification of Brucella spp., from cheese samples

Isolation and identification method of *Brucella* spp., were made according to Farrell (1974). The 3-tube MPN technique was used to determine the level of contamination of *Brucella* spp. in samples (BAM, 1998). Prepared in this way the two groups. The first groups of tubes incubated aerob condition; the second groups of tubes incubated %10 CO₂ condition for 5-7 day at 37°C (Gas generating kit Oxoid BR 038B). The Farrell's broth was prepared by adding 5% inactivated horse serum (Oxoid SR35), 10 g/l glucose (Merck 1.08346.1000) and 1 vial/500 ml of *Brucella* selective supplement (Oxoid SR83) to the *Brucella* broth (BBL 4311088). After incubation, 0.1 ml aliquot of each enriched homogenate was inoculated by the spread plating technique onto the Farrell's agar. Likewise prepared in this way the two groups. The Farrell's agar was prepared by adding 5% inactivated horse serum, 1 vial/500 ml of *Brucella* selective supplement to the *Brucella* Medium Base (Oxoid CM169). After incubation, colonies of suspect *Brucella* spp. were identified by their characteristic 1–2 mm diameter convex, entirely round edges, with translucent and pale yellow appearance and the colonies Gram stained, and examined for H₂S formation, urease, and catalase activities, and agglutination of antiserum using slide

agglutination tests (*Brucella abortus* antisera Difco 2871-47-7, *B. melitensis* antisera Difco 2889-47-7) and sensitivity staining.

Disc Diffusion Methods

Antibiotic sensitivity of isolates was performed according to disc diffusion susceptibility test (CLSI, 2008). The antibiotic discs (antibiotic concentration in µg) used consisted of streptomycin (10), trimethoprim/sulfamethoxazole (1.25/23.7), amoxicilline/clavulanic acid (20/10), lincomycine (2), neomycine (30), penicilline G (10), cephalixin (30),

ceftiofur (30), tetracycline (30), florphenicol (30), colistin (30) and danofloxacin (5).

RESULTS

According to Table 1 and Table 2, overall 14.2% of examined cheese samples were contaminated with *Brucella* spp. Twenty eight isolates were obtained from all samples. Among these 18 (9%) *Brucella abortus* and 10 (5%) of *Brucella melitensis* were identified.

Table 1. *Brucella* spp.'nin Peynir Örneklerindeki Dağılımı (%)

Table 1. Prevalance of *Brucella* Spp. in Cheese Samples (%)

Species of cheese	Sample (n)	<i>Brucella</i> spp. (%)	<i>B. melitensis</i>	<i>B. abortus</i>
Fresh white cheese	100	8	6	10
Tulum cheese	100	6	4	8
Total	200	14	10	18

Table 2. *Brucella* Spp.'nin Peynir Örneklerindeki Kontaminasyon Düzeyi (EMS/G)

Table 2. Contamination Levels of *Brucella* Spp. in Cheese Samples (NPN/G)

Number of isolates	Species of cheese	Level of contamination (MPN/g)	
		<i>B. melitensis</i>	<i>B. abortus</i>
2	Fresh white cheese		7.4 MPN/g
2	Fresh white cheese		7.4 MPN/g
6	Fresh white cheese		7.4 MPN/g
2	Fresh white cheese	3.0 MPN/g	
1	Fresh white cheese	3.0 MPN/g	
1	Fresh white cheese	3.0 MPN/g	
1	Fresh white cheese	3.6 MPN/g	
1	Fresh white cheese	3.6 MPN/g	
2	Tulum cheese	3.6 MPN/g	
1	Tulum cheese	3.6 MPN/g	
1	Tulum cheese	3.6 MPN/g	
4	Tulum cheese		6.1 MPN/g
2	Tulum cheese		6.1 MPN/g
2	Tulum cheese		6.1 MPN/g

Contamination level of *Brucella* spp. in most samples was detected as <3.6 MPN/g, but three tulum cheese samples and two white cheese samples 3.6 MPN/g, in three tulum cheese samples 6.1 MPN/g, in three fresh white cheese samples 7.4 MPN/g and in three fresh white cheese samples 3.0 MPN/g. *Brucella* spp. were detected in 6 samples (6%) of tulum cheese and 8 samples (8%) of fresh white cheese. *Brucella* spp. isolated from home-made

tulum cheese samples. In fresh cheese samples, were detected as 10 of them *B. abortus*, 6 of them were *B. melitensis*.

All isolates (12) which were obtained from tulum cheese samples were made at home. 8 of the isolates were identified as *B. abortus* and 4 of them *B. melitensis*. As a result of antibiotic tests applied to all isolates, it was determined that *B. abortus* and *B. melitensis* isolates were resistant to respectively;

streptomycin (77.7%) (80%), trimethoprim/sulfamethoxazole (77.7%) (100%), amoxicilline/clavulanic acid (100%) (100%), lincomycine (83.3%) (70%), neomycine (83.3%) (80%), penicilline G (100%) (100%), cephalixin (77.7%) (70%), ceftiofur (83.3%) (80%), tetracycline (100%) (100%), florphenicol (80%) (80%), colistin (100%) (100%) and danofloxacin (100%) (100%) (Table 3).

Table 3. İzolatların Antibiyotik Dirençlilik Profilleri
Table 3. Antibiotic Resistance Profile of The Isolates

Bacteria n/N: %	Streptomycin	Trimethoprim/ Sulfamethoxazole	Amoxicilline/ Clavulanic acid	Lincomycine	Neomycine	Penicilline	Cephalixin	Ceftiofur	Tetracycline	Florphenicol	Colistin	Danofloxacin
<i>B. abortus</i>	14/18 77.7	14/18 77.7	18/18 100	15/18 83.3	15/18 83.3	18/18 100	14/18 77.7	15/18 83.3	18/18 100	15/18 83.3	18/18 100	18/18 100
<i>B. melitensis</i>	8/10 80	10/10 100	10/10 100	7/10 70	8/10 80	10/10 100	7/10 70	8/10 80	10/10 100	8/10 80	10/10 100	10/10 100

DISCUSSION

Human brucellosis has long been recognized to be an occupation-related disease affecting primarily adult men engaged in some aspect of the livestock industry. Although this is true for *B. abortus* and *B. suis* infections, *Brucella melitensis* infection is primarily foodborne and associated with the consumption of unpasteurized dairy products (Chomel et al., 1994; Young, 1995). One outbreak due to sheep cheese caused by *B. melitensis* type 2 affected seven people after eating Italian Pecorino cheese made from unpasteurized milk (Galbraith et al., 1969). In addition to dairy products like cheese made from raw milk, cream, and butter, ice cream has also been reported to carry the risk of brucellosis. When the fresh materials are contaminated with *Brucella* spp., the end product will inevitably carry this risk (Anonymous, 2010; Bryan, 1983; Eyles, 1992).

In Turkey, although several investigations on the *Brucella* isolated from milk samples (Gürtürk et al., 1998; Beytut et al., 2002; Türütoğlu et al., 2003; Terzi, 2006), the studies on the isolation and contamination level of *Brucella* isolated from milk products are limited.

Cheese, particularly when prepared from untreated cows and sheep milk, was responsible for a wide range of infections. During 1983-1984 in Greece, 23 cases of brucellosis were associated with consumption of a variety of fresh home made

cheeses (Sharp, 1987). Sabbaghian (1975) informed that, out of 1220 fresh white cheese specimens 86 (7%) found infected with *Brucella melitensis* biotype I. Tantillo et al., (2001) reported that, a total of 46 cheese samples produced with sheep and goats milk assayed, and *Brucella* spp. detected in 46% of them, especially in cheese made from sheep milk. An another study, a total of 192 samples of illegal cheese collected from different regions of the states of São Paulo and Minas Gerais, Brazil. Among the 192 samples considered to be negative in *Brucella* spp. microbiological culture, PCR produced positive results in 29/141 (20.56%) samples of Minas frescal cheese and in 8/51 (15.69%) samples of cured Minas type cheese (Miyashiro et al., 2007). Haddad et al., 1993 reported that, 85 cheese samples were examined, 8 of which were positive for *B. abortus*. In Germany, human brucellosis was studied by analyzing national surveillance data (1965-2005) complemented by questionnaire based survey. It was indicated that, among cases with reported exposure risk, 59% were related to the consumption of unpasteurized cheese from brucellosis endemic countries (Dahouk et al., 2007). Akbarmehr (2011) reported that, 1000 cheese samples were collected from Sarab city. *Brucella* organisms were isolated from 22 samples (2.2%), seven of which (0.7%) were *B. melitensis* and the rest 15 positive samples (1.5%) were *B. abortus*. The result of Abbas and Talei (2010) showed that, out of three hundreds milk product samples collected only 9 *Brucella* isolates were found.

Researches which are performed in different regions of Turkey, show that various cheese contamination caused by *Brucella* spp. *Brucella* spp. was detected by Ünel et al (1968) 16% of white cheese in Balıkesir and district of Balıkesir; Mert (1984) 19.3% of white cheese collected in Ankara; Tunçbilek (1992) 5.2% of white cheese in Ankara. Patr and Dinçoğlu (2001), isolated *Brucella* spp. as 3.33% of 30 white cheese samples, 1.18% of 55 tulum cheese samples. Two samples of strains were *Brucella abortus* and *Brucella melitensis*, their levels were detected as 3.4×10^5 cfu/g and 4.6×10^2 respectively. Güllüce et al., (2003) informed that, *Brucella abortus* antigens in different cheese samples collected from Erzurum had been investigated by using enzyme-linked immunosorbent assay (ELISA). Totally 120 white cheese, 60 civil cheese and 52 lor cheese were analyzed and 26 of totally 120 white cheese were found to be *Brucella* positive. In civil and lor cheese samples no antigen of *Brucella* was encountered. Kasımoğlu (2002) reported that, a total of 105 samples including 35 raw milk, 35 cows' milk cheese, and 35 ewes' milk cheese samples were obtained from Kırkkale city and investigated for the presence and contamination level of *Brucella* species. According to analysis findings, *B. melitensis* was isolated from 5 (14.2%) of 35 ewes' milk cheese samples at the level of $3.6 \times 10(1)$ - $9.3 \times 10(3)$ MPN/g. *Brucella* spp. were not detected in any of raw milk and cows' milk cheese samples. Öngör et al., (2006) found in the analysis of 40 cheese samples collected from various markets, only *B. abortus* was detected by PCR using in two (5%) samples. Considering that with *Brucella* spp., contamination were either raw or not faced with enough heat process, in order to protect the public health especially in warm seasons considering inappropriate preparing and protecting condition. Thus, they can be suggested that in making butter, cream and whipped creamy pastry; pasteurised milk must be used and their preparation must be in hygiene and technical conditions (Taşçı and Kaymaz 2009). In our study, *Brucella* was found in tulum cheese and of fresh white cheese in which made from raw milk. Boiling of the milk resulted in killing large number of bacteria. The bacteria may be appear because of insufficient heat treatment. The bacteria not isolated from the some tulum and fresh white cheese because of inhibited the growth of bacteria using pasteurized milk.

In our study, *Brucella* spp. isolates were resistant (100%) to Amoxicilline/Clavulanic acid, Penicilline, Tetracycline, Colistin, florphenicol. Resistance to amoxicilline/clavulanic acid, penicilline, tetracycline, colistin and danofloxacin was evident for both *B. abortus* and *B. melitensis*. 4 out

of 18 *B. abortus* isolates susceptible streptomycin, trimethoprim/sulfamethoxazole and cephalixin; 3 out of lincomycine, neomycine, ceftiofur and florphenicol. 2 out of 10 *B. melitensis* isolates susceptible streptomycin, neomycine, ceftiofur and florphenicol; 3 out of lincomycine and cephalixin. This results is similar to that found by Abed Mohammed (1998) *Brucella* isolates are resistant to penicilline, nalidixic acid, bacitracin, polymixin B and lincomycine in percentage 100%, while Abbas and Talei (2010) showed that all isolated *Brucella* were sensitive to streptomycine, trimethoprim, gentamycine, rifampin, trimethoprim + sulamethaoxazok, kanamycine and tetracycline. Al-Abbasi et al. (1991) recorded similar results from ours that *Brucella* isolates are resistance to streptomycine and cotriamoxizol. Turkmani et al (2006) reported that, 17 *Brucella melitensis* isolates animal product, mainly milk. All the isolates were susceptible to tetracycline, streptomycine, gentamycine, ciprofloxacin, norfloxacin and levofloxacin.

Brucella isolates are generally considered susceptible to the recommended by the WHO antibiotics. Nevertheless sporadic cases of a kind of antibiotic resistance have been reported (Baykam et al., 2004; Lopez et al., 2004). Tetracyclines, rifampicin, trimethoprim-sulphamethoxazole (SXT), streptomycin, and other aminoglycosides, separately or in combinations, are most commonly used for brucellosis treatment (Hall, 1991; Pappas et al., 2005). Fluoroquinolones, and macrolides may serve as an alternative drug choice (Garcia et al., 1991; Quadri et al., 1995). In 1986, the WHO has released recommendations for use of doxycycline, combined with either rifampicin or streptomycin for treating human brucellosis. *Brucella* isolates are generally considered susceptible to the recommended by the WHO antibiotics. Nevertheless sporadic cases of a kind of antibiotic resistance have been reported (Baykam et al., 2004; Lopez et al., 2004).

Antibiotic resistant *Brucella* strains are rarely a cause of therapy failure (Hall, 1991). However, strains resistant to the main antimicrobial agents may emerge (Marianelli et al., 2004) and lead on to treatment inhibition.

CONCLUSION

Results of the study clearly indicated that local cheese (tulum cheese and fresh white cheese) produced from raw milk contaminated with *Brucella* spp. the presence of these pathogenic bacteria in raw milk cheeses or cheeses that become recontaminated after pasteurization pose a threat to human health

due to the increased number of cases and the severity of symptoms.

Globally, despite the remarkable results achieved by the majority of industrialized countries, where bovine brucellosis has been eradicated or controlled, small ruminant brucellosis remains a problem in some of these countries as well as in all developing countries. Basically, in developing countries, brucellosis is almost always present where small ruminants are kept. *B. melitensis* in cattle has emerged as an increasingly serious public health problem in some southern European countries and Israel as a result of the consumption of unpasteurized milk since *B. melitensis* is capable of colonizing the bovine udder.

In order to eliminate brucellosis in Turkey, an endemic region for the disease, precautions must be increased, the unregulated slaughtering and consumption of animals must be prevented, and the consumption of raw, unpasteurized milk and of dairy products made from such milk must be halted.

Prevention of brucellosis in humans still depends on the eradication or control of the disease in animal host, the exercise of hygienic precautions to limit exposure to infection through occupational activities, and the effective heating of dairy products and other potentially contaminated foods. Boiling or pasteurizing milk and milk product effectively kills brucellae in dairy products for human consumption. Furthermore; education assumes an important role in preventing the transmission of brucellosis from animals to humans.

The research entails for other antibiotic usage because of the requirement of drug therapy for animals and used to keep same drug for animal sick. Subsequently the antibiotic susceptibility testing of *Brucella* may help the choice of treatment in specific cases. Continued surveillance of emerging pathogens and its antimicrobial resistance patterns is warranted for milk and milk product to ensure a safe food and to implement effective treatments.

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