

Full Length Research Paper

The prevalence of *Brucella abortus* and *Brucella melitensis* in local cheese produced in Sarab city, Iran and its public health implication

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Brucellosis is an infectious disease caused by different species of *Brucella*. The main sources of human brucellosis for general population are dairy products prepared from raw milk. The aim of this study was to determine the prevalence of *Brucella abortus* and *Brucella melitensis* in unpasteurized cheese produced in Sarab city. For this purpose, a total of 1000 cheese samples were collected from Sarab city and its rural area from September 2006 till September 2007. Samples were cultured on selective *Brucella* agar and incubated at 37°C for a period of 2 weeks. *Brucella* species were confirmed by biochemical tests and then serological screening was performed by specific *Brucella* antisera. Overall, among 1000 samples which were examined by bacteriological tests, *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 prevalence rates of *Brucella* organisms in examined cheese samples in autumn, winter, spring, and summer were determined 2.42, 1.39, 2.5 and 2.62% respectively. This study revealed the public health hazard of local cheese produced in Sarab city.

Key words: *Brucella*, cheese, Sarab.

INTRODUCTION

Brucellosis is an infectious disease caused by different species of *Brucella*. These microorganisms cause disease in many different vertebrates. Pigs, cattle, goats, sheep and many kinds of wild animals are susceptible to *Brucella* infection (Bernard et al., 1990; Acha and Szyfres, 2003). The disease is a widespread zoonosis which is transmitted to man through direct contact with infected animals or through consumption of contaminated raw animal products, especially unpasteurized milk and soft cheese (Palmer et al., 1998; Seleem et al., 2010). The human brucellosis which is also called Undulant or Malta fever, is a serious public health problem and has been reported all over the world (Montanaro et al., 1992; Collier et al., 1998). In countries where eradication in animals is not feasible, prevention of human brucellosis is primarily based on food- safety measure, raising awareness and control of animal brucellosis. Most cases of human brucellosis are caused by *Brucella melitensis* and their geographical distribution follows very closely the distribution of ovine and caprine brucellosis (Palmer et al., 1998). The disease is prevalent on the southern and eastern edges of Mediterranean basin, particularly in

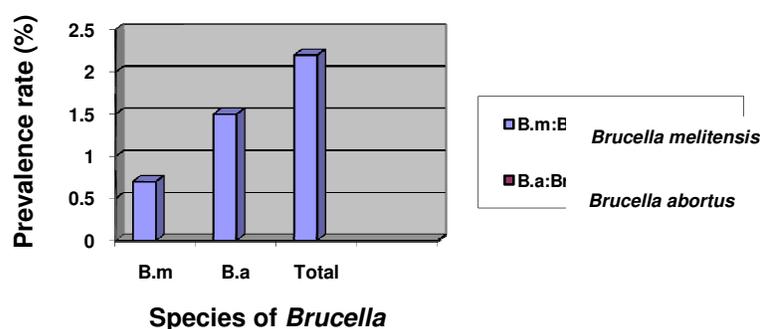
Tunisia, Libya, Egypt, Syria, and in the Arabian Peninsula and Iran. Human brucellosis is also common in many parts of north, central and South America, particularly in Mexico, Brazil, Colombia and Peru. New foci of human brucellosis have emerged particularly in central Asia (Pappas et al., 2006). In Iran, brucellosis is a notifiable disease. Despite the existing data about the disease, little has been documented about the prevalence of *Brucella* in dairy products in Iran. Sarab city, which is located in East Azarbaijan province, consists of 150000 inhabitants. This city is famous for its dairy products, and local unpasteurized cheese consumption is very popular in this region. The present research was designed with an objective to know the prevalence of *B. melitensis* and *B. abortus* in local cheese which is produced in Sarab city, and to show its public health implication.

MATERIALS AND METHODS

A total of 1000 cheese samples were collected randomly from Sarab city and its rural area, for a period of one year from September 2006 till September 2007. Each sample was included of

Table 1. The results of microbial examinations of the cheese samples collected from Sarab city in different season.

Season		Autumn	Winter	Spring	Summer	Total
Number of examined samples		165	286	320	229	1000
Positive samples of <i>Brucella</i> spp	Number	4	4	8	6	22
	Percent	2.42	1.39	2.5	2.62	2.2
Positive samples of <i>Brucella abortus</i>	Number	3	2	6	4	15
	Percent	1.81	0.69	1.87	1.74	1.5
Positive samples of <i>Brucella melitensis</i>	Number	1	2	2	2	7
	Percent	0.60	0.69	0.62	0.87	0.7

**Figure 1.** The prevalence of *B. abortus* and *B. melitensis* in examined cheese samples.

100 g of local cheese, of which, 10 g was diluted with 90 cc of normal saline to prepare suspension. Then, 0.1 cc of the suspension was cultured in brucella selective medium supplied with cyclohexamide. Because of slow growth of *Brucella*, incubation of samples was carried out for at least 2 weeks in the condition of 37°C with 5% Co₂. *Brucella* produces pinpoint, smooth, glistening and translucent colonies in selective serum agar (Quinn et al., 1994). After 2 weeks, if no growth was appeared in media culture, it was considered as a negative sample. For differentiation of different species of *Brucella*, biochemical tests were carried out based on H₂S production, urease activity, and growth of the microorganism in the presence of dyes. H₂S production was determined by lead acetate - strip method. Most strains of *B.abortus* have positive results in this test. Also, all strains of *Brucella* are urease positive except *B. ovis* and some strains of *B. melitensis*. The conventional test was carried out by incorporating the dyes, thionin and fuchsin separately, in trypticase soy agar at the concentration 1:50000. The dye was mixed with agar and poured into Petri dishes. The inoculated plates were incubated at 37°C under 5 to 10% CO₂ for 3 to 4 days and then examined for growth of *brucella*. Most strains of *B.abortus* are inhibited by thionin. Finally, serological screening was performed by specific *Brucella* antisera. So, identification of *Brucella* species were performed together with, colonial morphology, staining properties, biochemical tests and serological screening.

RESULTS

Out of 1000 samples which were examined by bacterial-

logical tests, *Brucella* organisms were isolated from 22 samples (2.2%), seven of which were *B. melitensis* (0.7%) and fifteen of the rest were *B. abortus* (1.5%). The rates of contamination of cheese samples with *B. abortus* in different seasons, autumn, winter, spring and summer were: 1.81, 0.69, 1.87 and 1.47% and for *B. melitensis*, were: 0.60, 0.69, 0.62 and 0.87% respectively. The results of microbial examination of cheese samples are showed in Table 1 and Figures 1 and 2.

DISCUSSION

Brucellosis is an infectious zoonotic disease which occurs naturally in animals and is transmitted to humans by direct or indirect routes such as consumption of unpasteurized dairy products. Most cases of infection caused by *B.abortus*, *B. melitensis*, *B. suis*, and *B. canis* arise from occupational or contact with infected animals. Farmers and their families, abattoir workers, butchers and veterinarians are particularly at risk (Palmer et al., 1998; Tabak et al., 2008). The main sources of human brucellosis for general population are dairy products prepared from unpasteurized milk and its derivatives (Sabbaghian et al., 1974). According to Table 1 and Figure 1, overall 2.2% of examined cheese samples were

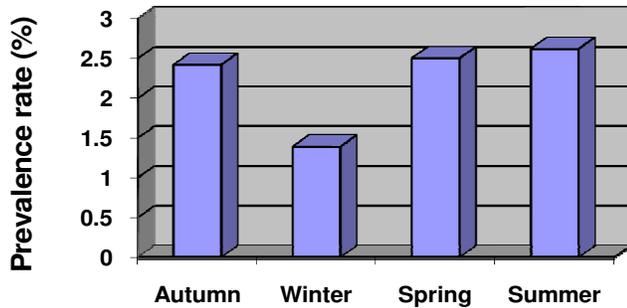


Figure 2. The prevalence of *B. abortus* and *B. melitensis* in examined cheese samples obtained in different season.

contaminated with *B. abortus* and *B. melitensis*. This result revealed an important and serious public health problem. Sheep and goats are the natural hosts for *B. melitensis* and cattle are the main reservoirs of *B. abortus* (Queen et al., 1994). The milk of infected animals may contain large numbers of viable organisms which become concentrated in products such as cheeses. Unpasteurized cheese has been recognized as a major vehicle of infection. *Brucella* spp. has been isolated from different dairy products all over the world (Eckman, 1975; Sancak et al., 1993; Hadad et al., 1997). Eyre et al. (1907) isolated *Brucella* spp. from raw cheese and ice cream. Stiles (1945) isolated *B. melitensis* from 16 different kinds of cheese samples. In another study which was carried out in Iraq, 85 cheese samples were examined, 8 of which were positive for *B. abortus* (Haddad et al., 1993). Sabbaghian et al. (1972) in Isfahan province of Iran isolated *B. melitensis* from 56 of 677 cheese samples and 1 of 160 cream samples. After an epidemiological study on human brucellosis, they also reported that raw dairy product consumption is the most probable source of *Brucella* infection in urban area. All of the above reports agree with our results and showed that raw cheese consumption should be emphasized as a public health hazard. A study which was carried out in Iran showed that, consumption of unpasteurized dairy products is significant risk factor for human brucellosis (Sofian et al., 2008). In Germany, human brucellosis was studied by analyzing national surveillance data (1965 to 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). Christos et al. (1999) reported that, consumption of cheese from pasteurized milk or consumption of cheese matured for over 3 months was found to be protective factor. Individuals of any age are susceptible to human brucellosis by consumption of contaminated dairy products. The disease may be acute or chronic form, and persists for years. Acute brucellosis may begin with fever, joint aches, malaise, and loss of appetite. Severe infection of human brucellosis can cause

a range of symptoms and injury of central nervous system, liver, and spleen occur (Collier et al., 1998). Sahin et al. (2009) reported a case of neurobrucellosis which complicated by optic obducens and vestibulocochlear nerve palsies due to *B. melitensis* infection. In a study which was conducted by Young and Suvannoparrat (1975), six cases of *B. melitensis* infections, that the common etiologic factor was, the ingestion of raw goat cheese, were reported, in which liver cell dysfunction and diffuse hepatitis was present in each case. Another conclusion of the present study was seasonal variation in the prevalence rate of *Brucella* in the examined cheese samples. As indicated in Figure 2, the highest rate of contamination occurred in spring and summer (2.5 and 2.62% respectively). This may be due to peak period of parturition among farm animals in spring. Finally, the findings of this research indicated that unpasteurized cheese is still an important source of human brucellosis in Iran, especially in East Azabayan province. Thus, pasteurization of milk and dairy products, education to avoid consuming unpasteurized milk and milk derivative, immunization of herds and elimination of infected animals should be highly recommended.

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