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DETERMINATION OF BRUCELLOSIS SEROPREVALENCE AND ASSOCIATED RISK FACTORS IN AMASYA PROVINCE

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Abstract

It is important to determine the current seroprevalence by investigating Brucellosis disease, which is an important cause of morbidity, in the general population. In this study, it was aimed to determine brucellosis seroprevalence and associated risk factors in the population residing in the city center of Amasya. The population of this study consisted of the population aged fifteen and over living in the city center of Amasya. For the cross-sectional study, it was aimed to reach 464 people with the multi-stage sampling method. As a cluster unit, villages representing rural areas and family health centers representing neighborhoods in urban areas were taken. The data of the study carried out between the years 2021-2023 were collected between 16/05/2022-22/02/2023. After the individuals included in the sample were informed about the purpose of the study and the procedures to be applied, a questionnaire form prepared by the researchers

was applied to those who agreed to participate. Following the application of the questionnaire, venous blood sample was taken and specific immunoglobulins were investigated by Brucellacapt test in serum sample. Statistical significance level was accepted as p<0.05 for all tests. Brucellosis seroprevalence was found to be 0.8% as a result of the study. It was determined that 4 seropositive cases were between the ages of 48-78 and all of them resided in rural areas (p=0.025). It was determined that 263 (55.6%) of the participants had not heard of Brucellosis before. The rate of being aware of Brucellosis disease was higher in people living in rural areas and having a high school or higher education level (p=0.028 and p=0.002, respectively). While 103 (49.0%) of the people who heard of brucellosis disease did not know any symptoms of the disease, the number of people who stated that they consumed food made from raw or unboiled milk in the last year was found to be 244 (51.3%). Considering that the disease is transmitted by aerosol from the barns where the sick animals are located, in addition to direct contact, it is necessary to inform the individuals dealing with animal husbandry to take adequate precautions. The fact that the rate of being aware of brucellosis is lower among those living in urban areas and those with secondary school education or below indicates that more educational studies on the disease should be carried out by targeting these segments in particular.

Key Words: Brucellosis, Seroprevalence, Risk Factors

Özet

Önemli bir morbidite sebebi olan Bruselloz hastalığının toplum genelinde araştırılarak güncel seroprevalansın tespit edilmesi önem taşımaktadır. Bu çalışmada Amasya ili merkezinde ikamet eden nüfusta Bruselloz seroprevalansı ve ilişkili olduğu risk faktörlerinin belirlenmesi amaçlanmıştır. Bu çalışmanın evrenini Amasya il merkezinde yaşayan on beş yaş ve üzeri nüfus oluşturdu. Kesitsel tipteki çalışma için çok aşamalı örnekleme yöntemi ile 464 kişiye ulaşılması hedeflendi. Kırsal kesimde köyler, kentsel kesimde ise mahalleleri temsilen aile sağlığı merkezleri küme birimi olarak alındı. 2021-2023 yıları arasında yürütülen çalışmanın verileri 16/05/2022-22/02/2023 tarihleri arasında toplandı. Örnekleme dahil edilen bireyler

çalışmanın amacı ve uygulanacak işlemler konusunda bilgilendirildikten sonra katılmayı kabul edenlere arastırmacılar tarafından hazırlanan anket formu uvgulandı. Anketin tamamlanmasından sonra venöz kan örneği alınarak serum örneğinde Brucellacapt testi ile spesifik immünoglobulinler araştırıldı. İstatistiksel anlamlılık düzeyi tüm testler için p<0,05 olarak kabul edildi. Çalışma sonucunda Bruselloz seroprevalansı %0,8 olarak bulundu. Seropozitif 4 olgunun 48-78 vas aralığında olduğu ve tamamının kırsal kesimde ikamet ettiği belirlendi (p=0,025). Katılımcıların 263'ünün (%55,6) daha önce Bruselloz hastalığını duymadığı belirlendi. Kırsal kesimde yaşayan ve lise ve üzeri öğrenim düzeyine sahip kişilerde Bruselloz hastalığını duyma oranı daha yüksekti (sırasıyla p=0,028 ve p=0,002). Bruselloz hastalığını duyan kişilerden 103'ü (%49,0) hastalığın hiçbir belirtisini bilemezken, son bir sene içerisinde çiğ veya kaynatılmamış sütten yapılmış gıda tükettiğini belirten kişi sayısı ise 244 (%51,3) olarak bulundu. Hastalığın doğrudan temasın yanında, hasta hayvanların bulunduğu ahırlardan aerosol yoluyla bulaştığı da göz önüne alınırsa, hayvancılıkla uğraşan bireylere yeterli önlemleri almaları yönünde bilgilendirme çalışmaları yapılması gerekmektedir. Kentsel bölgelerde yaşayanlarda ve ortaokul ve altında öğrenim düzeyine sahip olanlarda Bruselloz hastalığından haberdar olma oranının daha düşük olması özellikle bu kesimlerin hedeflenerek hastalık hakkında daha fazla eğitim çalışması yapılması gerektiğini göstermektedir.

Anahtar Kelimeler: Bruselloz, Seroprevalans, Risk faktörleri

1. Introduction

Brucellosis is a significant public health concern that can be transmitted from infected animals to humans, causing both human and animal health issues as well as economic losses (El-Sayed and Awad, 2018). Brucella bacteria are small, gram-negative, non-motile, non-spore-forming, coccobacilli with dimensions ranging from 0.6 to 1.5 μ m. In developed countries where control measures against animal brucellosis have been implemented, the frequency of human cases has significantly decreased (SB, 2014). However, this zoonotic disease is still widespread in various regions worldwide, including Latin America, Africa, the Mediterranean, the Middle East, and Western Asia (CDC, 2017). Among the species that commonly cause infection in humans, *B. abortus* affects cattle, *B. melitensis* affects sheep and goats, *B. suis* affects pigs, and B. canis affects dogs, foxes, and jackals. Although *B. abortus* has a broad geographic distribution, *B. melitensis* is the species responsible for the highest number of human cases (Corbel, 2006).

Transmission of the disease to humans occurs through various routes, including the consumption of unpasteurized milk and dairy products, direct contact with infected animal tissues due to occupational reasons, and inhalation of contaminated aerosols. Occupations requiring close contact with animals have a higher seropositivity rate (Corbel, 2006). Diagnosis can be made by isolating the microorganism from clinical samples or by demonstrating an antibody response through serological methods. The bacterium can be isolated from various clinical samples, primarily blood and bone marrow cultures (Yagupsky et al., 2019). Culture is the gold standard for diagnosis, and the isolation of the bacterium confirms the diagnosis. However, the sensitivity of cultures can be affected by factors such as the stage at which the sample is taken from the patient, the type of culture medium used, and whether treatment has started before sample collection. For these reasons, serological methods have gained importance in the diagnosis of the disease and are commonly used. With the current serological tests, it is possible to diagnose over 95% of cases, but the appropriate combination of tests is crucial. These combinations often involve a test that detects agglutinating antibodies consisting of total IgM, IgG, and IgA (such as Rose Bengal and Standard Tube Agglutination) and a test that detects nonagglutinating antibodies that typically occur in the late stage of the disease (such as Coombs IgG or ELISA) (SB, 2014). Recently, a rapid and easily applicable "immunocapture" agglutination test that provides results within 24 hours has also been used (Casanova et al., 2009).

Awareness and knowledge about brucellosis are insufficient worldwide, particularly in Asian and African countries (Zhang et al., 2019). Areas with intensive livestock farming, where the disease can spread from infected animals to humans, are at higher risk. According to data from the Ministry of Health, a total of 6,457 confirmed cases were reported in Türkiye in 2017, and the incidence rate, which was 13.7 per 100,000 population in 2008, decreased to 7.9 per 100,000 population in 2017. In the same year, the incidence rate of brucellosis in Amasya province was calculated as 2.4 per 100,000 population (SB, 2019). According to the Turkish Statistical Institute (TÜİK) data for 2021, Amasya has a total of 485,835 large and small livestock animals. With 145 animals per 100 people, Amasya exceeds the Turkish average in animal production (TUİK, 2021a). Therefore, it is important to conduct research on the disease's prevalence in the general population and to determine the associated risk factors in our region. This study aims to determine the seroprevalence of brucellosis and its related risk factors in the population residing in the city center of Amasya province.

2. Material and Methods

The study population consisted of 118,991 individuals aged fifteen and over residing in the city center of Amasya. With 80% power and 5% type-1 error, the minimum sample size was calculated as 238 when the Brucellosis seroprevalence in the population was taken as 2.9% (Turhan et al., 2010) and the deviation was 5%. Since cluster sampling method was used for the cross-sectional study, it was aimed to reach a minimum of 464 individuals by multiplying it with a design effect of 1.95. The sample was stratified by (i) place of residence (rural/urban), (ii) gender, and (iii) age groups. The determination of strata was based on the 2020 Turkish Statistical Institute (TÜİK) population results (TUİK, 2021b). A multi-stage sampling method was employed to reach individuals to be included in the study. Villages in rural areas and neighborhoods in urban areas were taken as cluster units, representing family health centers. Five villages and four family health centers were included in the study using a simple random sampling method.

The data of the study were collected between May 16, 2022, and February 22, 2023. In the rural stratum, individuals aged fifteen and over who were present at home during the visit hours of randomly selected households and, in the urban stratum, individuals aged fifteen and over who applied to family health centers for any reason on visit days were included in the study. After informing the individuals to be included in the study about the purpose of the research and the procedures to be applied, a questionnaire prepared by the researchers was administered. The questionnaire consisted of 42 questions, including a section on socio-demographic characteristics consisting of 10 questions, a professional data consisting of 12 questions, and a section investigating the history, knowledge, and attitude towards brucellosis consisting of 20 questions. Since a part of the questionnaire. Face-to-face interviews were conducted by the researchers in both regions. After completing the questionnaire, a 5 mL venous blood sample was taken, and the serum was stored at -20 °C under cold chain conditions within three hours of collection.

The Brucellacapt test (Vircell, Spain) is a Coombs microplate test based on agglutination that detects total antibodies for the diagnosis of brucellosis. The tests were conducted following the manufacturer's recommendations. Initially, a screening procedure was applied to serum samples at a 1/320 dilution. In the screening test, 795 µl of diluent was added to 5 µl of serum, and after dilution, 50 µl was transferred to the test well (coated with anti-human immunoglobulins). Then, 50 µl of Brucella antigen suspension was added to the well. After incubation at 37 °C for 24 hours, the results were evaluated. If a layer completely covered the surface of the well, the test was recorded as negative. Positive samples were further diluted up to 1/5120, and the test was repeated. Titers of 1/320 and higher were considered significant positive. Positive and negative controls were added for each plate. Individuals with positive Brucellacapt test results were referred to the Department of Infectious Diseases for further investigation.

2.1. Ethical Approval

Ethical approval was obtained from Amasya University Non-interventional Clinical Research Ethics Committee (Date: 06.07.2021 - Number: 22661).

2.2. Statistical Analysis

After coding the data obtained from the research, they were transferred to a computer and analyzed using SPSS 22.0 package program. Normally distributed continuous variables were expressed as mean ± standard deviation, non-normally distributed numerical variables as median (interquartile range), and categorical data as number (%). The normality of continuous variables was evaluated using the Kolmogorov-Smirnov test. A p-value of <0.05 was considered statistically significant for all tests.

3. Results

The median age of the 476 participants was 53 years (interquartile range: 38-63), and 253 (53.2%) of them were female. It was determined that 286 participants (60.1%) resided in urban areas, 212 (44.9%) had primary school education, and 278 (58.6%) were employed in income-generating jobs (Table 1).

| Gender | n | % |
|-------------------------|-----|------|
| Male | 223 | 46.8 |
| Woman | 253 | 53.2 |
| Age group (years) | | |
| 15-18 | 23 | 4.8 |
| 19-24 | 22 | 4.6 |
| 25-44 | 133 | 27.9 |
| 45-64 | 191 | 40.1 |
| 65 and over | 107 | 22.5 |
| Residential area | | |
| Rural | 190 | 39.9 |
| Urban | 286 | 60.1 |
| Education level* | | |
| Illiterate | 29 | 6.1 |
| Primary school | 212 | 44.9 |
| Middle school | 37 | 7.8 |
| High school | 77 | 16.3 |
| Undergraduate and above | 117 | 24.8 |
| Working status* | | |
| Working | 278 | 58.6 |
| Unemployed/housewife | 84 | 17.7 |
| Retired | 73 | 15.4 |
| Student | 39 | 8.2 |
| Number of households* | | |
| 1 | 33 | 7.4 |
| 2 | 130 | 29.1 |
| 3 | 97 | 21.7 |
| 4 and above | 186 | 41.7 |

Table 1. Distribution of sociodemographic characteristics of the participants

*It does not give the total number due to missing data. Percentages were calculated based on valid answers.

Among the employed individuals, 93 (33.4%) were engaged in cattle or small livestock farming. It was found that 75 of these individuals (80.6%) did not use gloves during contact with animals, 92 (98.9%) had their animals vaccinated against Brucella, 7 (7.5%) had experienced disease-related deaths in their animals in the past year, and 11 (11.8%) had experienced miscarriages in their animals. Additionally, 83 (89.2%) consumed the milk from their own

animals, and 42 (50.6%) did not boil all dairy products (Table 2). All participants reported that cheese was the dairy product for which the boiling process was not performed.

| Gloves during contact with animals | n | % |
|--|-----|------|
| Never used | 75 | 80.6 |
| Continuous user | 12 | 12.9 |
| Sometimes use | 6 | 6.5 |
| Animal Brucella vaccine | | |
| Made by | 92 | 98.9 |
| Not having it done | 1 | 1.1 |
| Animal deaths due to disease in the last y | ear | |
| Yes | 7 | 7.5 |
| No | 86 | 92.5 |
| Miscarriage in the animal in the last year | | |
| Yes | 11 | 11.8 |
| No | 82 | 88.2 |
| Consuming own animals' milk | | |
| Consuming | 83 | 89.2 |
| Non-consumptive | 10 | 10.8 |
| Boiling own animals' milk* | | |
| Do not boil all dairy products | 42 | 50.6 |
| Boiling all dairy products | 41 | 49.4 |

Table 2. Distribution of health behaviors and animal health stories of participants engaged in animal husbandry (n=93)

* It does not give the total number due to missing data. Percentages were calculated based on valid answers.

Among the participants, 263 (55.6%) had never heard of brucellosis. The distribution of those who were aware of brucellosis according to socio-demographic characteristics is presented in Table 3. The rate of awareness of brucellosis was higher among those living in rural areas and those with a high school education or higher (p=0.028 and p=0.002, respectively). Among those who were aware of brucellosis, 103 (49.0%) did not know any symptoms of the disease, while 67 (31.9%) mentioned that the disease can be transmitted from both animals and dairy products.

| Gender | n | %* | \mathbf{p}^{\dagger} |
|-------------------------|-----|------|------------------------|
| Male | 109 | 48.9 | 0.064 |
| Woman | 101 | 40.4 | _ |
| Residential area | | | |
| Rural | 96 | 50.5 | 0.028 |
| Urban | 114 | 40.3 | _ |
| Education level | | | |
| Middle school and below | 107 | 38.5 | 0.002 |
| High school and above | 101 | 52.6 | _ |
| Job | | | |
| Animal producer | 59 | 63.4 | 0.125 |
| Other professions | 96 | 52.5 | _ |

Table 3. Distribution of participants who are aware of brucellosis according to their sociodemographic characteristics

* Percentage of rows

† Compared with the group that had not heard of brucellosis. Pearson chi-square test

Among the dairy products consumed daily, cheese was the most consumed, with 269 participants (56.5%). This was followed by butter (48.5%), yogurt (46.8%), and milk (8.8%). A total of 244 participants (51.3%) reported consuming food made from raw or unpasteurized milk in the past year. All participants referred to this dairy product as cheese.

The consumption of food made from raw or unpasteurized milk in the past year did not show significant differences based on gender, type of residence, education level, awareness of brucellosis, and occupation (p>0.05).

Only 7 participants (1.5%) reported previously being diagnosed with brucellosis, but all of these cases had a negative result on the Brucellacapt test. The median time between the date of diagnosis and the date of data collection for these cases was 3 (2.6) years.

Within the scope of the study, 4 participants (0.8%) tested positive on the Brucellacapt test. All of these seropositive cases were in the 48-78 age range and lived in rural areas (p=0.025). Other characteristics of these individuals are presented in Table 4.

| Gender | n | %* | \mathbf{p}^{\dagger} |
|---|---|-----|------------------------|
| Male | 2 | 0.9 | 1.000 |
| Woman | 2 | 0.8 | |
| Residential area | | | |
| Rural | 4 | 2.1 | 0.025 |
| Urban | 0 | 0.0 | |
| Education level | | | |
| Middle school and below | 4 | 1.4 | 0.147 |
| High school and above | 0 | 0.0 | |
| Job | | | |
| Animal producer | 3 | 3.2 | 0.117 |
| Other professions | 1 | 0.5 | |
| Consumption of unboiled dairy products in the last year | | | |
| Yes | 3 | 1.3 | 0.354 |
| No | 1 | 0.4 | |
| Brucellosis symptoms in the last year | | | |
| Yes | 3 | 1.1 | 1.000 |
| No | 1 | 0.5 | |

Table 4. Distribution of various characteristics of participants seropositive for brucellosis

*Percentage of rows

+Compared with individuals seronegative for brucellosis. Fisher's exact test

4. Discussion

Brucellosis is a zoonotic disease, and one of the riskiest occupations for its transmission is animal husbandry. Given that nearly half of the participants in this study living in rural areas in the center of Amasya province are engaged in animal husbandry, their knowledge and attitudes regarding brucellosis, which can be transmitted directly from animals or through dairy products, are of great importance. It was found that only 19.4% of individuals engaged in animal husbandry used gloves during contact with animals. In a study conducted in Adana, the rate of glove use among those engaged in animal husbandry was found to be 36.1% (Şahin et al., 2019). In a study in Kars, this rate was 19.6% (Akkuş et al., 2011), and in Samsun, glove use during milking was reported to be 37.5% (Kaygisiz and Özkan, 2021). As seen in this study and in various regions of

Türkiye, the use of gloves during contact with animals is quite low. It is known that brucellosis can be transmitted through damaged skin during contact with infected animals (Haque et al., 2011). Therefore, educating individuals engaged in animal husbandry is crucial to break the transmission chain of the disease. Another measure for preventing brucellosis is the vaccination of animals. In Türkiye, *B. melitensis* Rev.1 vaccine is used for sheep and goats, and *B. abortus* S19 vaccine is used for cattle. It has been shown that these live attenuated vaccines provide protection between 80-100% (Çakır and Yıldırım, 2018). In this study, all but one of the animal breeders had their animals vaccinated against brucellosis. In studies conducted in various provinces in Türkiye, the rate of animal breeders vaccinating their animals against brucellosis varies between 49.9% and 81.8% (Tavşan et al., 2015; Avcı et al., 2017; Alkan et al., 2022). The higher vaccination rate in this study may be attributed to the Free Vaccine Supply and Application Protocol signed between the Ministry of Agriculture and Forestry and the Union of Turkish Municipalities as part of the program to combat animal diseases and pests in February 2022 (TBB, 2022). Payments made to breeders for the vaccines applied may have increased the vaccination rate.

One of the striking findings of the study was the inadequacy of knowledge about brucellosis. Only 44.4% of the participants stated that they had heard of brucellosis before. In studies where the sample consists of individuals living in rural areas, this rate varies between 65.2% and 98.5% (Avci et al., 2017; Babaoğlu and Demir, 2017; Alkan et al., 2022). Although the rate of awareness of brucellosis among participants living in rural areas in this study was lower than in the literature, it was higher than that of the urban area stratum. This can be attributed to the fact that people living in rural areas are more involved in animal husbandry and are more informed about the precautions to be taken. The other group with high awareness of the disease was individuals with a high school education or higher. In a study by Babaoğlu and Demir, it was also observed that the rate of awareness of brucellosis increased as the level of education increased, but 41.6% of those who knew about the disease could not mention any symptoms (Babaoğlu and Demir, 2017). In this study, too, almost half of those who were aware of the disease could not mention any symptoms. This situation indicates that awareness-raising efforts for the disease should be conducted more effectively.

Among the dairy products consumed daily, cheese was the most consumed by participants, at 56.5%. Similar findings have been reported in studies conducted in various regions of the country,

where individuals most commonly consumed cheese daily (Engindeniz et al., 2021; Alkan et al., 2022). The wide variety of cheese available in Türkiye and its frequent consumption at breakfast make this result expected. Brucella bacteria can survive in fresh cheese made from unpasteurized or unboiled milk for 15-100 days (SB, 2019). In this study, 51.3% of the participants reported consuming food made from raw or unpasteurized milk in the past year, and all of them referred to this product as fresh cheese. The frequency of consuming cheese made from raw milk varies between 39.7% and 95.5% in Türkiye (Tavşan et al., 2015; Avcı et al., 2017; Babaoğlu and Demir, 2017; Alkan et al., 2022). Regardless of variables such as education level and awareness of brucellosis, the high frequency of fresh cheese consumption in this study suggests that interventions should be made to promote healthy eating habits in the fight against the disease.

The number of individuals who previously claimed to have been diagnosed with Brucellosis was found to be 7 (1.5%). All of these individuals tested negative for the Brucellacapt test. The fact that IgM antibodies, which develop after Brucellosis, can only be detected in 8.3% of individuals two years after the onset of the disease, and that IgG becomes negative within the first year of the disease in the absence of incomplete treatment or chronicity, can be attributed to this situation (Avijgan et al., 2019). In our study, among individuals who claimed to have had the disease before, the shortest time between the diagnosis date and the start of the study was two years, confirming this situation. The number of individuals with a positive Brucellacapt test result was 4 (0.8%). According to community-based studies on the subject, Brucellosis seroprevalence was determined to be 0.9% in Sanliurfa and 1% in Kahramanmaras, using the Rose-Bengal (RB) and Standard Tube Agglutination (STA) tests with a sample from the population in the city center (Murat et al., 2011; Tavsan et al., 2015). In a village in Isparta, Brucellosis seroprevalence was 3.6% using STA (Sözen and Gönen, 2012), and in a study involving individuals residing in both urban and rural areas, Brucellosis seroprevalence in Hatay was determined to be 2.9% based on STA test results (Turhan et al., 2010). When the literature is examined, it is observed that as the representation rate of the rural population in the sample increases, Brucellosis seroprevalence also increases. A similar situation is observed in our study, where all seropositive cases were found to reside in rural areas. This is an expected outcome since individuals living in rural areas are more likely to engage in animal husbandry. In this study, both urban and rural populations were included, so we believe that the most accurate seroprevalence value for comparison can be

found in the study conducted in Hatay. Accordingly, the Brucellosis seroprevalence in Amasya city center was lower than that in the Hatay study, as well as lower than the other three studies. To explain this difference, the most accurate approach is to examine the difference in sensitivity and specificity of the tests. According to the most recent data, when the SAT test is used alone, its sensitivity and specificity are 74.4% and 95.2%, respectively, while when the Brucellacapt test is used alone, these criteria are 88.3% and 100%, respectively (Xu et al., 2023). In light of this data, it can be suggested that the Brucellacapt test is a better measurement tool, and the relatively low seroprevalence in our study may be due to the low specificity of the SAT test used in other studies. In this study, it was observed that the only variable significantly affecting seropositivity for Brucellosis was the residential area, while other sociodemographic variables such as gender and education level did not have an impact. Similar findings have been observed in studies conducted in various regions of Turkey (Turhan et al., 2010; Murat et al., 2011; Tavşan et al., 2015).

One of the significant limitations of this study is its relatively small sample size, which resulted in a limited number of positive cases and prevented the identification of risk factors affecting Brucellosis seroprevalence through statistical analysis such as logistic regression. However, despite this limitation, this study is valuable as it is the first community-based study to determine Brucellosis seroprevalence in Amasya city center.

5. Conclusion

In conclusion, the seroprevalence of Brucellosis in Amasya city center was found to be 0.8%. The results indicated a higher seroprevalence in rural residents, with seroprevalence being similar among different socio-demographic variables. It was observed that a significant proportion of those involved in animal husbandry did not use gloves while handling animals, which may explain the higher seroprevalence in rural areas. Considering that Brucellosis can also be transmitted through aerosols from barns where infected animals are present, it is crucial to inform individuals involved in animal husbandry about taking adequate precautions. Another significant finding is that more than half of the participants were not aware of Brucellosis, and this lack of awareness was higher in urban areas and among individuals with middle school or lower education levels. Therefore, it is essential to conduct more educational campaigns targeting these groups. Although the consumption of unpasteurized dairy products in the last year did not

affect Brucellosis seroprevalence in this study, it is known that fresh dairy products like cheese pose a higher risk for Brucellosis. Efforts should be made to discourage the consumption of such dairy products and promote the use of pasteurized or boiled dairy products.

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