Presence of *Coxiella burnetii* in cheese samples from sheep farms in Northeast Anatolia, Turkey

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SUMMARY

Coxiella burnetii is a zoonotic bacterial pathogen responsible for causing Q fever in humans. It is commonly found in livestock, particularly sheep, posing a potential risk of transmission to humans through various sources. Understanding the prevalence of *C. burnetii* in different matrices, such as cheese, is essential for evaluating the potential public health implications. This study aimed to determine the prevalence of *C. burnetii* in cheese samples obtained from sheep farms in Northeast Anatolia, Turkey, and investigated the effects of *C. burnetii*-positive cheese samples on pH, water activity (a_w), and fat and salt percentages. Ninety cheese samples were gathered from 23 sheep farms within the study region, with 5.6% (5/90) of the Turkish white brined cheese samples testing positive for *C. burnetii*. The pH values of the positive and negative samples did not significantly differ ($p > 0.05$), with mean values of 5.07 ± 0.38 and 5.18 ± 0.79, respectively. However, a statistically significant difference ($p < 0.05$) was observed in the fat percentages between positive (22.4 ± 3.44%) and negative (28.5 ± 1.57%) cheese samples. No significant differences were found in the a_w and salt percentages between the positive and negative cheese samples. This study presents the first molecular evidence of *C. burnetii* in Turkish white brined cheese, highlighting the potential contamination of this popular dairy product. These findings underscore the importance of implementing effective control measures in sheep farming and dairy production to minimize the risk of *C. burnetii* transmission to humans. Further studies are warranted to investigate the sources, routes, and potential public health implications of *C. burnetii* in dairy products and its role in the epidemiology of Q fever in Turkey.

KEY WORDS

Dairy microbiology; *DNA*; microbiology; raw milk; white brined cheese.

INTRODUCTION

Q fever is one of the most common zoonotic diseases globally, and farm animals are its main infectious agents or reservoirs. While this disease has a wide clinical spectrum in humans, including asymptomatic, acute, chronic, and fatal, it is mostly asymptomatic in animals. Infected animals shed the disease agent in their milk, feces, placental membranes, and abortion fluids. The main routes of transmission of Q fever to humans are inhalation of contaminated aerosols and consumption of unpasteurized milk or dairy products (1, 2)

Coxiella burnetii (*C. burnetii*) is a compulsory intracellular bacterium that is difficult and dangerous to isolate in a standard laboratory environment. Direct diagnosis of infection with it is rarely made as it requires working in a biosafety level 3 laboratory with experienced personnel. However, the presence of antibodies directed against *C. burnetii* can be shown with serological tests, such as enzyme-linked immunosorbent assay (ELISA). Because ELISA has 82-100% sensitivity and 93-96% specificity for small ruminants, it is preferred over other serological methods, especially for herd screening (1). The polymerase chain reaction (PCR) test, one of the molecular methods used to detect the DNA of bacteria, is accepted as a suitable tool for detecting *C. burnetii* (2).

Unpasteurized milk and dairy products prepared with raw milk are consumed in many parts of the world. Many kinds of high-quality cheese with gastronomic value are also made from unpasteurized raw milk around the world (3-5). *C. burnetii* can maintain its vitality in cheese and is a risk factor for Q fever (5). Several studies have investigated its presence in different cheese products (3-7). Turkish white brined cheese, one of the most essential traditional cheeses produced in Turkey, whose annual production represents more than 60% of the country's total cheese production (8), is made by small family businesses using traditional methods, and is produced without heat treatment (9). The survival or growth rates of pathogenic bacteria in cheese depend on conditions such as water activity (a_w), pH, and salt content (10).

The present study aimed to determine the prevalence of *C. burnetii* in cheese samples obtained from sheep farms in the Agri, Erzurum, and Erzincan provinces in Northeast Anatolia, Turkey, where sheep farming is intense. The effects of *C. burnetii*-positive cheese samples on pH, water activity (a_w), and fat and salt percentages were also investigated.

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MATERIAL AND METHODS

The current study was approved by Atatürk University ethics committee decision numbered 2018/66. This study was carried out with 23 sheep herds in the provinces of Northeast Anatolia in Turkey, namely Agri, Erzurum, and Erzincan (Figure 1). The sample size was calculated based on the instructions provided on the website http://sampsize.sourceforge.net/, within a 22.6% estimated prevalence and a 95% confidence interval. A total of 90 Turkish white brined cheese samples (250 g) were obtained from growers (30 from each province). The samples were then transported to the laboratory under a cold chain (4±1°C) for laboratory analysis.

The commercial DNA extraction kit DNeasy Blood & Tissue Cador Pathogen Mini Kit (Qiagen, Hilden, Germany) was used to extract DNA from the cheese samples. Before the analysis of the samples, samples in which *C. Burnetii* positive control DNA was spiked into a previously determined positive cheese sample and a negative cheese sample were used for test optimization for internal extraction control. The analyzed DNAs were produced by applying touchdown PCR according to the method recommended by a previous study (11). A transposase gene region specifically found in the genomic material of *C. burnetii* was used. The Nine Mile strain and ultrapure water were used as positive and negative controls, respectively. The obtained amplicons were identified in 1.5% agarose gel via electrophoresis. Agarose gel was placed in the trans illuminator, and imaging was performed under ultraviolet light using the Geneline program. *C. burnetii* was considered present if amplicons were detected in the agarose gel with a 687 bp DNA fragment.

The pH value of the homogenate cheese was measured with a pH meter (Orion 3 Star pH Benchtop, Thermo Scientific, USA). The a_w of the cheese samples was determined using an a_w meter (Aqua LAB 4TE, Meter Group, USA). The fat and salt percentages were detected using the modified Gerber method (12) and the Mohr method (13), respectively. A T-test was performed for physicochemical analysis. The significance threshold was $p < 0.05$. The data are presented as mean ± standard deviation. All the data were analyzed using the SPSS statistical package (IBM, Version 20.0, SPSS Inc., USA, 2018).

RESULTS

Out of the 90 Turkish white brined cheese samples analyzed, 5 samples (5.6%) tested positive for the presence of *C. burnetii*. Figure 2 illustrates the PCR reactions of these positively identified cheese samples. The pH values for both the positive and negative samples were recorded, with the positive samples exhibiting a mean pH of 5.07 ± 0.38 and the negative samples having a mean pH of 5.18 ± 0.79; the difference in pH values between the two groups was not statistically significant ($p > 0.05$). On the other hand, the fat percentages in the positive samples averaged 22.4 ± 3.44%, while the negative samples had a higher mean fat percentage of 28.5 ± 1.57%, and this difference was

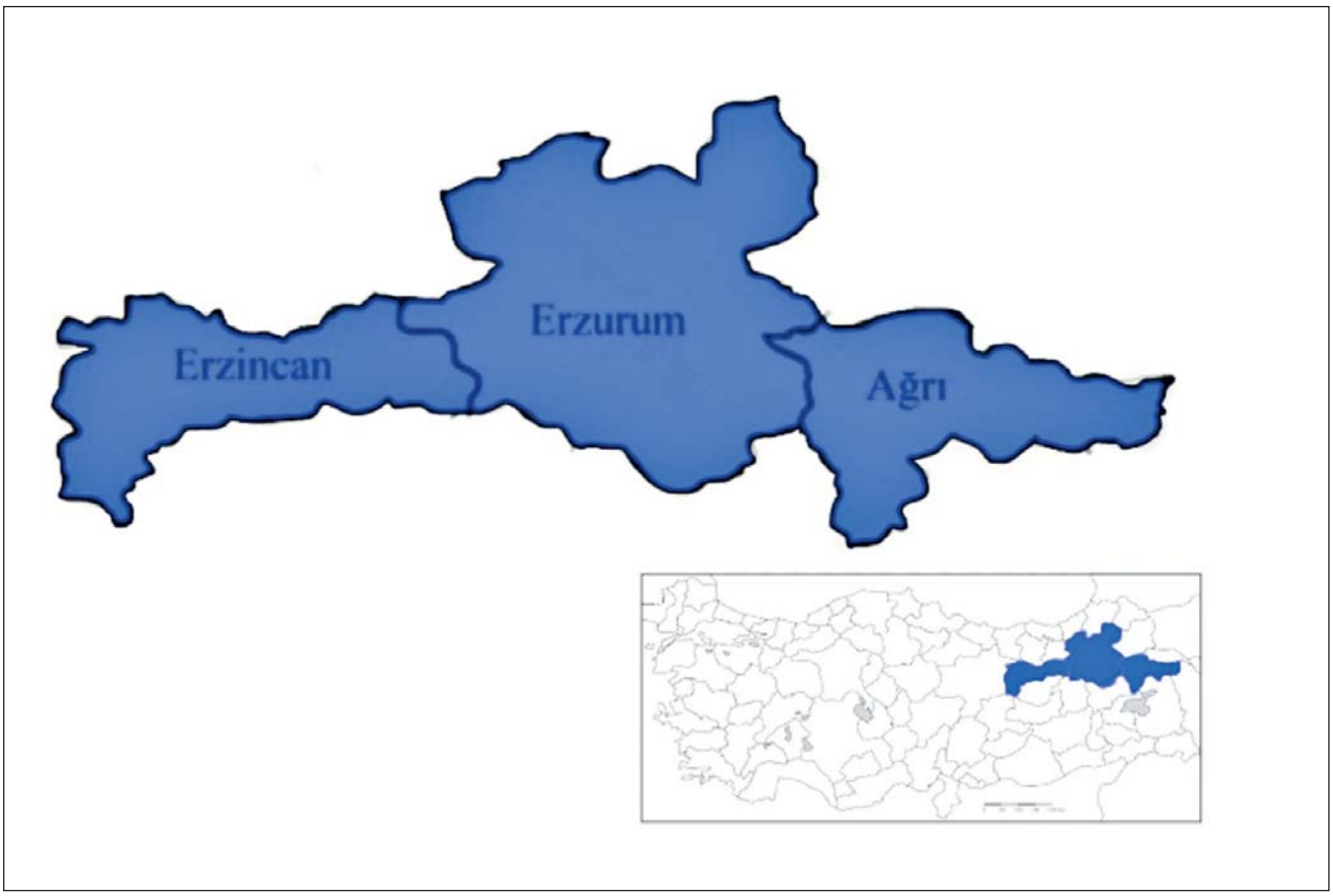

Figure 1 - Map showing the provinces of Agri, Erzurum, and Erzincan located in Northeast Anatolia, Turkey.

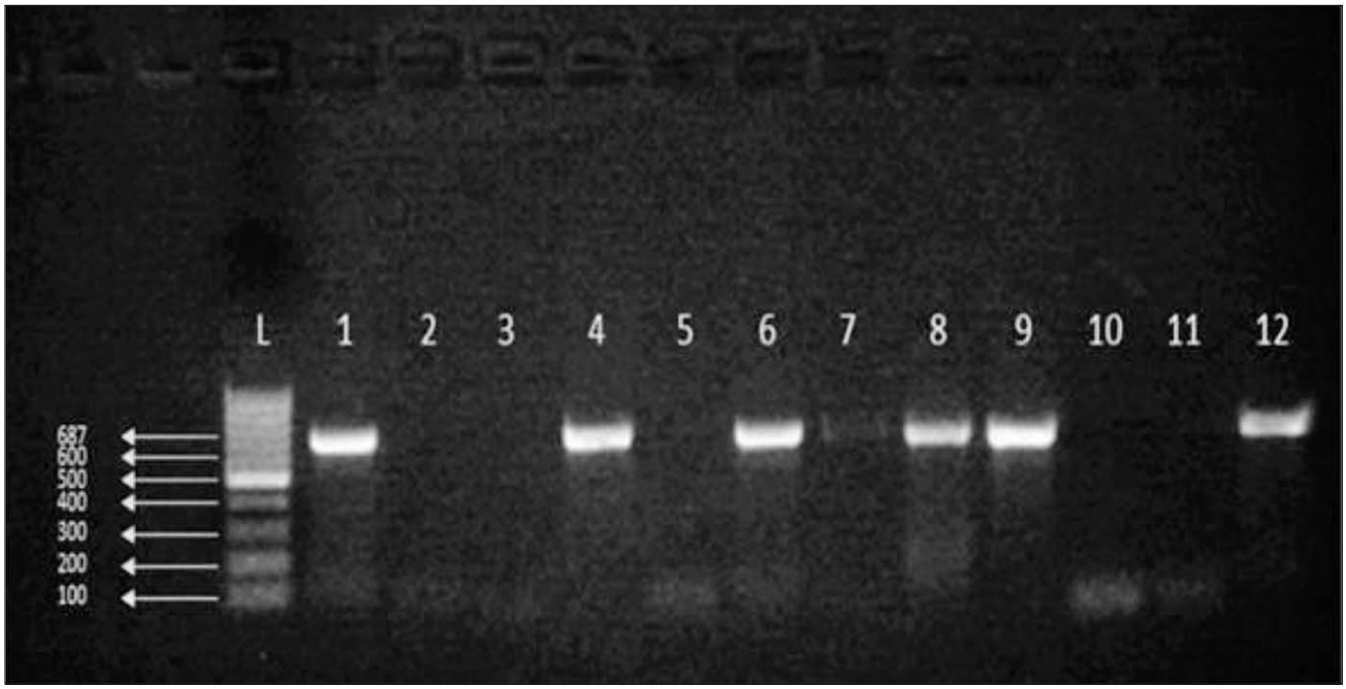

Figure 2 - Image of *C. burnetii*-positive cheese samples' polymerase chain reactions. In the agarose gel image of the *C. burnetii*-positive cheese samples, the reference indicator (L = ladder, 100-1000 bp), 1st-place positive control, 2nd-place negative control, and 4th, 6th, 8th, 9th, and 12th places were 687 bp bands belonging to the positive samples.

found to be statistically significant ($p < 0.05$). However, no significant disparities were observed in the a_w and salt percentages between the positive and negative samples, as detailed in Table 1.

DISCUSSION

The objective of this study was to assess the prevalence of *C. burnetii* in cheese samples obtained from sheep farms situated in the Agri, Erzurum, and Erzincan regions of Northeast Anatolia, Turkey. While several investigations have explored the presence of *C. burnetii* in cheese samples across different countries (3-7), it is important to note that our study represents the pioneering effort in conducting such research within Turkey. In our study, we discovered that the *C. burnetii* DNA positivity rate in Turkish white brined cheese was 5.6%. A study conducted in Brazil reported a positivity rate of 9.43% among 53 cheese samples (14). Furthermore, an investigation in Spain revealed a significantly higher positivity rate of 29.9% among 67 samples (5). In a study conducted in Italy, the presence of *C. burnetii* DNA was observed in 32.14% of the 84 cheese samples that were analyzed (3). A broader study conducted in Italy encompassed the analysis of 169 cheese samples. This comprehensive investigation yielded an overall prevalence of *C. burnetii* of 21.3%. Importantly, this prevalence exhibited variations among different cheese species (6). Furthermore, a study carried out in Japan identified *C. burnetii* DNA in 28 out of 147 cheese samples (7), providing valuable insights into the prevalence of this pathogen in cheese products within the Japanese context. The study conducted in Iran, where *C. burnetii* was detected in only 2 out of 46 cheese samples, resulting in a positivity rate of 4.35% (4), closely resembles the findings of our current study. This similarity in positivity rates may be attributed to the geographic proximity of the Northeast Anatolia region of Turkey, where our study was conducted, to Iran.

A pH value of 5.07 was obtained in the cheese samples with *C. burnetii* DNA positivity in the present study. This finding was compatible with the 5.11 pH obtained in the *C. burnetii* positive cheese samples in a previous study (5). In the present study, the a_w values of the *C. burnetii* positive and *C. burnetii* negative cheeses were 0.9504 and 0.9304, respectively. These were in agreement with the a_w values in a previous study (5), which ranged from 0.9688 to 0.9040. It has been shown that the pH value and a_w in *C. burnetii* positive cheese samples may affect the viability of the agent during the ripening process in cheese (5). While it remains uncertain whether the *C. burnetii* within the analyzed cheeses is viable in this study, the viability of *C. burnetii* in these examined cheeses can be ascertained through culture tests and quantified using q-PCR. These considerations may be regarded as limitations of the current study. Nevertheless, based on the results of the present study, it can be said that lower pH and a_w values in *C. burnetii* positive cheese samples do not affect the DNA of *C. burnetii*.

Table 1 - Mean values of pH, water activity (a_w), fat percentage (%), and salt percentage (%) in the 90 white cheese samples obtained from 23 sheep farms in Northeast Anatolia, Turkey.

Molecular results	n	pH	a_w	fat (%)	salt (%)
Positive	5	5.07 ± 0.38	0.95 ± 0.03	22.4 ± 3.44*	4.38 ± 1.31
Negative	85	5.18 ± 0.79	0.93 ± 0.03	28.5 ± 1.57*	4.40 ± 2.94

Values marked with * were found significant at the $P < 0.05$ level. n: Number of samples

In the present study, the fat percentage of *C. burnetii* positive Turkish white brined cheese was found to be 22.4% ($P < 0.05$). The *C. burnetii* genome encodes the enzymes necessary for the synthesis of fatty acids and phospholipids. In addition to fatty acids and their derivatives, *C. burnetii* may have reduced the fat ratio in cheese due to the need for isoprenoids and sterols because the parasitic vacuole membrane is rich in cholesterol (15).

Previous study reported that the salt ratios of the *C. burnetii* positive cheeses in their study were 7.14-8.16% (3). In the present study, the average salt ratio in the *C. burnetii* positive cheeses was 4.37%, and no relationship was observed between *C. burnetii* positivity and salt ratio. The salt ratio of cheeses is related to the duration of brining and the amount of salt used in it; thus, the difference in the salt ratios of the cheese samples in the present study might have been due to these variables. One way of transmitting zoonotic agents to humans is through the consumption of contaminated foods (4). The presence of *C. burnetii* in cheeses offered for people's consumption poses a long-lasting risk to public health because it has been reported that the bacteria can survive in cheeses for almost 9 months (5). Because *C. burnetii* is one of the most heat-resistant pathogens (16), raw milk must be effectively pasteurized before using it to produce dairy products. The current study has a limitation related to the complexity of nucleic acid extraction from matrices like cheese, owing to the high fat content. It would have been more suitable to employ a food-specific extraction kit instead of a generic one.

CONCLUSION

Although the present study was conducted with sheep cheeses made with unpasteurized milk, the study's findings can also be applied to other kinds of cheese and other dairy products made with raw milk, regardless of ruminant type. The detection of *C. burnetii* in cheeses available in local markets seems to indicate a public health risk at both the production and consumption stages. For this reason, efforts should be made to apply routine control methods based on vaccination and to carry out biosafety practices to obtain milk and dairy products that do not contain *C. burnetii*.

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Author Contributions

Berna Yanmaz: Conceptualization; Formal analysis; Investigation; Methodology; Visualization; Writing - original draft. Ziya Gokalp Ceylan: Conceptualization; Investigation; Methodology; Writing - review & editing.

Conflict of Interest

The authors declare no conflict of interest.

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