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Evaluation of Culture and Real Time PCR Methods for the Diagnosis of Brucellosis

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ABSTRACT

This study aimed to determine the presence of *Brucella* spp. in 73 abortion materials collected from various provinces of Turkey. Culture isolation and Real-time PCR methods were used for this purpose, and the laboratory diagnosis was compared. The clinical samples were taken from aborted cattle. A total of 79 samples were used for this study, consisting of 28 fetal stomach contents and 51 vaginal swabs. The presence of *Brucella* spp. was investigated using both culture isolation and real-time PCR methods. Of the collected samples, eight (10.12%) were found to be positive for *Brucella* spp. through culture isolation. These same samples were then subjected to Real Time PCR testing for comparison. Thirteen point nine two percent (11/79) of the samples tested positive for *Brucella* spp. using real-time PCR. This suggests that inhibitors, bacterial load in clinical samples, and possible contamination may reduce the chance of isolating the bacteria in culture or lead to false negative results. Therefore, it can be concluded that real-time PCR is a fast and reliable alternative to culture for diagnosing brucellosis.

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INTRODUCTION

Brucellosis is an important and widespread zoonotic disease in terms of public health, causing economic losses in Turkey and in the world. This zoonosis, which affects our country, continues to be updated within the scope of one health. In the eradication of brucellosis, it is very important to immediately intervene in the diseased area and diagnose it. In this way can be protected human and animal health and risks can be minimized. One of the diagnostic methods used in diagnosing the disease of brucellosis, isolation from culture is a time-consuming, difficult method that can give false negative results. Therefore, molecular methods have become increasingly important nowadays (Probert, 2004).

Brucellosis is caused by gram-negative aerobic coccobacilli, also known as facultative intracellular bacteria. Identification of *Brucella* spp. involves culture isolation, biochemical tests, serological tests, and molecular methods (Baysal, 1989; Güzelant, 2009; Jimenez, 2004; Qasem, 2015). This process is time-consuming and tedious, and the culture isolation stage poses a high risk of infection for laboratory workers (Qasem, 2015). In humans, brucellosis can present with a range of clinical symptoms. Acute cases may exhibit fever, myalgia, myocarditis, and pancytopenia (Özenci, 1984; Qasem, 2015; Ulaş, 2012). The disease can progress to a chronic state, affecting various organs and tissues, particularly the joints (Probert, 2004).

To overcome these problems, nucleic acid amplification remains up-to-date for rapid detection of *Brucella* and validation of tests. Alternative methods such as real time PCR, which provides rapid and reliable diagnosis in brucellosis eradication, have always started a desired process (Probert, 2004).

Brucellosis diagnosis involves culture isolation, serological tests, and molecular studies. Although serological tests are commonly used, they may not be sufficient for early-stage diagnosis and may not differentiate between active and inactive infections due to cross-reactions (Diaz, 2020; Ko, 2012). Culture isolation is considered the gold standard for brucellosis diagnosis. The most commonly used methods are those described by Elfaki (2005). However, when attempting to isolate brucellosis from culture, contamination with flora is a frequent occurrence, particularly in environmental samples. Given the laboratory conditions, high-risk factors require increased attention, as noted by Yagupsky (1999).

For this reason, different diagnostic methods have been developed. For the diagnosis of brucellosis, DNA-based methods such as polymerase chain reaction (PCR) are used especially for confirmation. There are few reports on the comparison of these tests. The aim of the current study is to compare the gold standard culture method, which is frequently used in different brucellosis tests, with the real time PCR method.

The aim of this study is to determine the presence of Brucellosis disease, which has a high zoonotic importance, by culture and real-time PCR. At the same time, these methods will be evaluated comparatively.

MATERIAL AND METHOD

The ethical permission was ensured from The Local Ethics Committee code of 2021/1 and 17.02.2021.

Study Area and Sample Collection

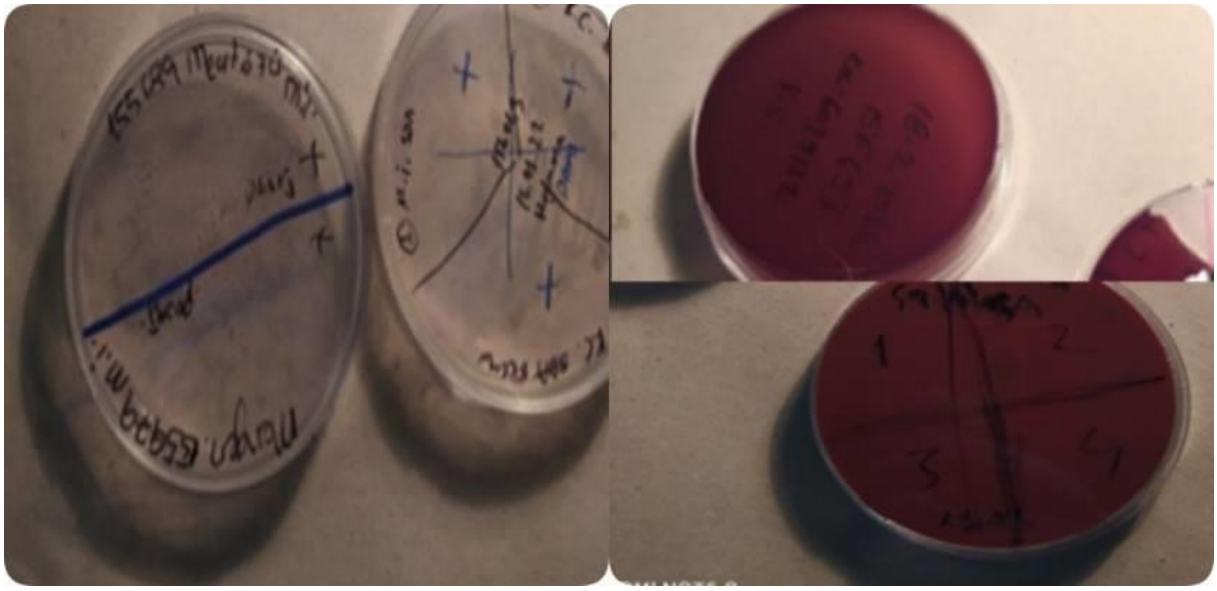
The study was conducted in Central Anatolia and the Western Black Sea province of Turkey. A total of 79 clinical materials were collected from different settlements in Turkey, including 28 fetal stomach contents and 51 vaginal swabs from aborted cattle patients. The clinical samples were stored at -20°C for culture analysis and RT-PCR.

Culture Analysis

Skirrow agar and SDA were used for culture isolation of *Brucella* spp. After inoculation, the medium was incubated at 37°C with a microaerophilic environment for 1-3 weeks. After the culture isolation, the species started to breed from the 3rd day and daily controls were provided. Colony morphologies in the passages of the samples were observed (Kurtoğlu, 2014; Marouf;2021). Confirmed by Gram stain, biochemical tests oxidase tests. catalase and urease activity (Mena-Bueno, 2021; Yagupsky, 2022). After isolation, macroscopic morphology, incubation time and biochemical properties of colonies were tested to confirm *Brucella* spp (Mena-Bueno, 2021). Successful colonies obtained were stored in stock solution at -80°C (Yagupsky, 2022).

Figure 1.

Image of Culture Isolation Media for Brusella spp. (SDA and Skirrow Medium)



Real Time PCR

When performing DNA extraction from our materials, it was prepared in accordance with the manufacturer's instructions. Nucleic acid purification kit (QIAampDNA Mini Kit, Qiagen, Germany) was used for this procedure.

The primer and probe sequence used for the TaqMan real-time PCR assay for identification of *Brucella* spp. It targets the bcsp31 gene (Sabour et al, 2020).

The conditions of the real-time PCR test were performed as follows: 40 cycles of 95 °C for 10 minutes, 95 °C for 20 seconds, and 56 °C for 45 seconds. Oligonucleotide primers and probes used in real-time PCR method to detect *Brucella* spp agent in our samples; Forward primer:

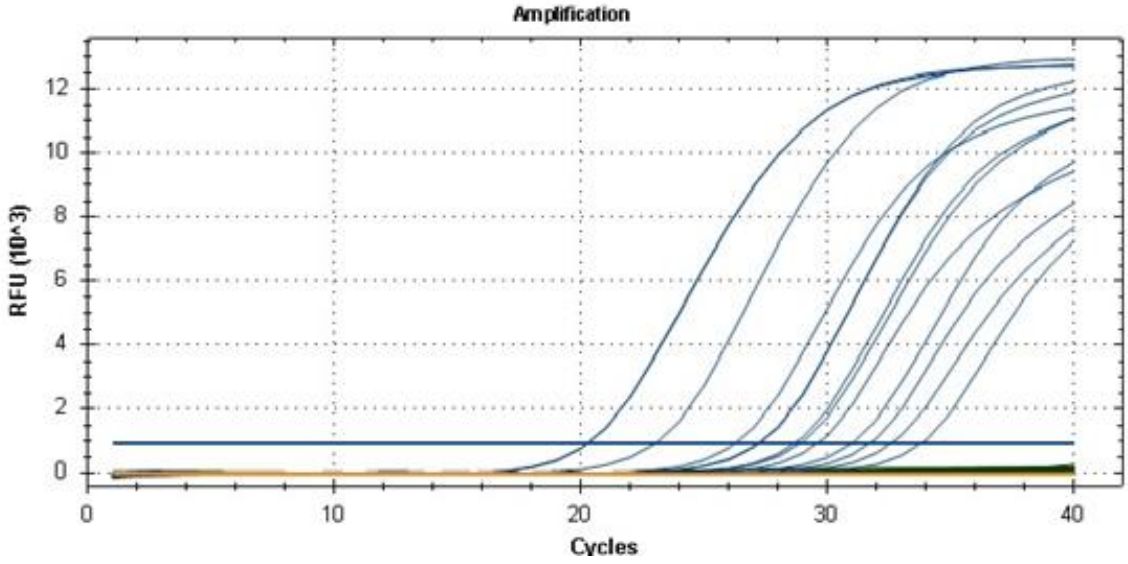
GTCCGGTTGCCAATATCAATGC, Reverse primer: GGGTAAAGCGTCGCCAGAAG Reverse: AAATCTTCCACCTTTGCCCTTGCCATCA (Sabour et al, 2020).

RESULTS

After isolating clinical materials from culture, it was found that eight (10.12%) samples contained *Brucella* spp. In order to eliminate Salmonella spp and Campylobacter spp, inoculations were made on MacConkey agar and Skirrow agar, but the results were negative. Real Time PCR test method identified 11 (13.92%) samples as *Brucella* spp (Figure 2).

Figure 2.

Brusella spp. Amplification Curves of Real Time PCR Reactions of Clinical Materials



DISCUSSION

Control and eradication methods are crucial for both animals and humans. Vaccination and eradication of farm animals are particularly important. Vaccines are highly effective in immunizing farm animals against diseases. The control method used for brucellosis in our country and around the world is vaccination of animals. However, animal movements and uncontrolled contact of breeders with farm animals can lead to the spread of diseases. Due to its zoonotic nature, effective antibiotic treatment for humans is often challenging when dealing with brucellosis (Hoffman, 2016). Therefore, controlling animal brucellosis and, quick and accurate diagnosis of the disease is crucial in preventing human infection.

Traditional bacteriology methods can be very demanding and long-lasting (Probert, 2004). Various media such as Columbia blood agar, Serum dextrose agar, skirrow agar, tryptone soy agar, serum dextrose agar and *Brucella* medium base are used to isolate *Brucella* species (De Miguel, 2011). In our routine laboratory, *Brucella* spp. we get effective results from especially two media that we use in the diagnosis of the disease. Therefore, *Brucella* spp. serum dextrose agar (SDA) (Oxoid) and Skirrow agar (Oxoid) were used to detect the causative agent. All samples were processed under Biosafety Level Two (BSL-2). Particularly high personal security procedures have been implemented. In addition to these measures, staff training, decontamination, record keeping, emergency procedures and risk assessments have been completed (Schuring, 1991). Successful isolation of *Brucella* spp.

from clinical samples may vary depending on the level of environmental contamination and bacterial load. Therefore, it is important to pay special attention to potential contaminants when working in laboratory conditions to increase the chances of successful isolation. In our study, *Brucella* was successfully isolated from eight clinical samples. The real-time PCR results for the same samples were positive. Furthermore, the samples were evaluated for *Salmonella* and *Campylobacter* species using the culture method, but all samples tested negative. In addition to the eight samples that tested positive in culture, three more samples (two vaginal swabs and one fetal stomach content) were found to be positive for *Brucella* spp by real-time PCR. Based on the anamnesis and positive real-time PCR results, these three animals were classified as 'probable patients' due to abortion.

Today, molecular methods are frequently used. Among these methods, Real-Time PCR method, sensitivity, amplification efficiency, results in a short time, it is known to be more advantageous than other methods (Bounaadja, 2009; Probert, 2004). Culture analysis and real-time methods were compared in research on humans and animals at different studies (Dal, 2019). In these comparisons, sensitivity and specificity studies were carried out. In most studies by researchers PCR was observed to be more effective than isolation from culture (Abedi, 2020; El-Diasty, 2016). On the other hand, real time PCR played a more effective role in detecting the presence of DNA.

Our research compared the results of the real-time PCR method with the culture method, confirming that real-time PCR is a viable alternative for diagnosis, as previously shown in other studies (Awwad, 2016; Mukherjee, 2015; Navarro, 2002). Given the high cost and difficulty of vaccination/culling studies against this infection, which is currently prevalent in our country, accurate diagnosis is critical for effective control. Fast and sensitive diagnostic tools are crucial for developing an emergency protection and control strategy against brucellosis, particularly during disease outbreaks when animals are experiencing abortions (Mukherjee, 2015). Additionally, this is a preventive measure for public health within the framework of One Health.

As a result, as emphasized in the research articles, it was observed in my study that the real time PCR test was very fast and reliable as a method in the diagnosis of the agent. In the light of all this information, real-time PCR can be used as an alternative method to isolation from culture, which has disadvantages in terms of time control and workload in the diagnosis of brucellosis disease.

Ethical Statement

A section of this study was presented as Oral Presentation and Abstract Paper at the Second International Congress on Biological and Health Science on 24.02.2022.

Ethics Committee Approval

17/01/2021 dated 17/02/2021 and 2021/01 numbered was given by Veterınary Control Central Research Instıtute Local Ethics Committee

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Conflict of Interest

Authors declare that there is no conflict of interest.

Sustainable Development Goals (SDG): 3 Good Health and Well-Being

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