ORIGINAL ARTICLE

Molecular Detection of *Coxiella burnetii* as a Potential Cause of Abortion in Iraq

Azhar J. Kadhim*, Zainab D. Degaim
Department of Microbiology, College of Medicine, University of Thi-Qar, Thi-Qar 64001, Iraq

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****Corresponding Author:***
Azhar Jawad Kadhim
Department of Microbiology, College of Medicine, University of Thi-Qar, Thi-Qar 64001, Iraq
azhar.j.msc@utq.edu.iq

ABSTRACT

Background: *The purpose of the present research was to identify one of the potential causes of abortion in Iraq—Coxiella burnetii (C. burnetii).* ***Methodology:*** *The case-control study included 200 blood samples were collected from women who had experienced spontaneous abortions at two hospitals in Thi-Qar province during the period from October to November 2024. Similarly, 100 blood samples were collected from pregnant women who underwent either a normal vaginal delivery or a caesarean section, serving as the control group. DNA was extracted from samples and analyzed using a real-time PCR approach targeting the IS1111 gene to detect the presence of C. burnetii.* ***Results:*** *The data indicated that the highest percentage of women who had abortions were in the age group of 26–35 years (47%), while the lowest percentage was observed in the 36–44 years' age group (20%). There was no statistically significant difference in abortion rates based on residency, with approximately 51.0% of cases from urban areas and 49.0% from rural areas. Regarding gestational age, 54.0% of abortions occurred during the first trimester, while the lowest rate (18.0%) was reported in the third trimester. Real-time PCR results showed that only 8 out of 200 DNA samples collected from women who had experienced abortions (4%) tested positive for the IS1111 gene, indicating infection with C. burnetii.* ***Conclusion:*** *RT-PCR is a critical and accurate method for detecting C. burnetii in aborted women who are not diagnosed in hospitals in Thi-Qar province, Iraq.*

INTRODUCTION

Abortion is the cessation of pregnancy prior to the fetus's viability outside the uterus[1]. It has emerged as a critical health issue among women of reproductive age. Various factors contribute to miscarriage, including infections caused by microorganisms such as bacteria, viruses, and parasites[2]. Certain intracellular bacteria are known to be toxic to embryonic tissues, resulting in severe complications for both the mother and the fetus. *Brucella*, *Listeria monocytogenes*, *Chlamydia trachomatis*, and *Coxiella burnetii* are among the microorganisms implicated[2].

C. burnetii is an obligate intracellular pathogen, measuring 0.2–0.4 µm in width and 0.4–1.0 µm in length. It is classified as Gram-negative due to the characteristics of its cell membrane, despite not being stainable with the Gram stain[3]. *C. burnetii* is the causative agent of a global zoonosis known as Q fever. This pathogen invades monocytes and macrophages, replicating within acidic phagolysosomes and evading host defenses through several immune evasion mechanisms, primarily attributed to the structure of its lipopolysaccharide[4]. *C. burnetii* organisms have been identified in aborted human placentas. [5] Placental infections with *C. burnetii* are most prevalent during the first and second trimesters of pregnancy[6]. Infected pregnant women face an increased risk of miscarriage, stillbirth, preterm delivery, low birth weight, fetal death, and congenital anomalies such as omphalocele, hypospadias, Potter syndrome, congenital hydronephrosis, syndactyly, and growth retardation[7].

The *IS1111* gene is a standard insertion sequence, characterized as a genetically compact bacterial transposable element encoding only a transposase (Tnp)—an enzyme that facilitates its own transposition[8]. Insertion sequences like *IS1111* are considered "selfish" DNA elements that can autonomously replicate and transpose to various loci within and between bacterial genomes[8].

Molecular techniques such as conventional PCR and quantitative PCR (qPCR), which target the *IS1111* gene, are widely recognized as highly sensitive methods for the specific detection and quantification of *C. burnetii*[9]. The *C. burnetii* genome contains multiple copies of *IS1111* scattered throughout the chromosome[10,11]. The presence of these repetitive elements contributes to rapid genomic evolution, as recombination among *IS1111* copies facilitates significant genomic plasticity in *C. burnetii*, including chromosomal rearrangements and large-scale insertions and deletions[11].

This study aims to investigate one of the potential causative agents of abortion in Iraq—*C. burnetii*—in women who have experienced miscarriage.

METHODOLOGY

Participants and study design

The case-control study included two hundred blood samples from women who had undergone abortions were collected. Blood was drawn from each participant using disposable syringes in EDTA tubes at two hospitals in Thi-Qar province: Al Haboby General Hospital and Bint Al-Huda General Hospital, during the period from October to November 2024. Additionally, one hundred blood samples were collected from pregnant women who underwent either normal vaginal delivery or caesarean section, serving as the control group. Blood specimens were stored at -20 °C until DNA extraction for the detection of C. burnetii using the real-time PCR technique.

Extraction of genomic DNA

The chromosomal DNA from whole blood samples of both aborted women and the control group was extracted using the gSYNC™ DNA Extraction Kit, according to the manufacturer's guidelines.

RT-PCR identification of *C. burnetii* via *IS1111* gene

The amplification of the *IS1111* gene in *C. burnetii* was, forward: 5'-AAAACGGATAAAAAGAGTCTGTGGTT and reverse: CCACACAAGCGCGATTCAT. Conducted using a realtime PCR thermocycler system (Setratagene system, USA). The final volume of the RT-PCR reaction tubes was 20 µl, comprising 12.5 µl of Syber Green Master Mix, 1 µl each of forward and reverse primers specific to the gene, 3 µl of DNA template, with the remaining volume adjusted using nuclease-free water. The realtime PCR protocol for the IS1111 gene involved an initial denaturation at 95°C for 10 minutes, succeeded by 45 cycles comprising denaturation at 95°C for 15 seconds and annealing at 60°C for 60 seconds[12].

Ethical Approval:

The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki. The committee of researchers at the Thi-Qar Health Directorate (No. 2024/235 on 22/10/2024) has viewed and approved this study. The informed consent was obtained from all participants.

Statistical Analysis

The IBM SPSS 26 version was used.

RESULTS

The current results showed that the most aborted women in second age group (26-35 years; 47%) followed by the age group (16-25 years; 33%), while the lowest age groups of aborted women were detected in the age group (36-44years; 20%). There was a significant difference at (p. value <0.001) among age groups, as shown in Table (1).

Table 1: Prevalence of aborted women according to age groups

Age group	No. (%)	*p*. value
16-25	66 (33.0%)	*p*. value < 0.001
26-35	94 (47.0%)	
36-44	40 (20.0%)	
Total	**200 (100%)**	

The current results showed a non-significant difference at (p. value <0.05), according to residency, there was recorded (51.0%) of women in urban resident and (49.0%) in rural resident, as in Table (2).

Table 2: Prevalence of aborted women according to residency

Residency	No. (%)	*p*. value
Rural	98 (49.0%)	*p*. value 0.777
Urban	105 (51.0%)	
Total	**200 (100%)**	

The results of abortion history showed that the aborted women increased significantly at (p. value <0.001) in the first semester (54.0%), followed by second semester (28.0%); while, the lowest percentage at the third semester (18.0%), as listed in table (3).

Table 3: Prevalence of aborted women according to history of abortion

History of Abortion	No. (%)	*p*. value
1st	108 (54.0%)	*p*. value < 0.001
2nd	56 (28.0%)	
3rd	36 (18.0%)	
Total	**200 (100%)**	

Diagnosis of *C. burnetii* by real time PCR technique

Out of the 200 abortion samples, only (8/200) of total DNA samples extracted from aborted women (4%) gave a positive result of *IS1111* gene that used to detect *C. burnetii*, which indicates the importance of the diagnosis of this bacterium. While, the *IS1111* gene was not detected in control group, Figure (1) represented the amplification plot of *IS1111* gene.

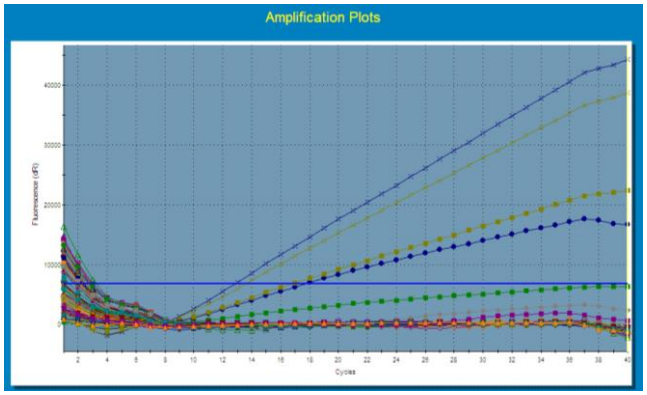

Fig. 1: Amplification plot of *IS1111* gene in *C. burnetii*

DISCUSSION

The results of this study showed that the highest percentage of abortions occurred among women in the 26–35 years' age group (47%). According to the current findings, the incidence of *Coxiella burnetii* in samples taken from women who had experienced abortions was 4% (8 out of 200 samples). Miscarriage is a common pregnancy complication that may occur frequently or infrequently[13]. Intracellular bacteria have been proven to damage embryonic cells and cause serious disorders in both mothers and fetuses[2]. In recent years, a significant number of abortion cases in Iraq have been attributed to *C. burnetii*. However, limited investigations have been conducted on Q fever infections in pregnant women and the associated adverse and abnormal outcomes. One major mechanism of pregnancy complications associated with Q fever is vascular thrombosis, which leads to placental insufficiency and miscarriage. Research also indicates the possibility of direct fetal infection[14].

The present study also found no statistically significant difference in abortion rates based on residency, with 51.0% of affected women residing in urban areas and 49.0% in rural areas. Serological studies of *C. burnetii* in pregnant women living in rural areas with direct animal contact have shown prevalence rates of 29.3% in parts of southwestern and northern Iran and up to 48.4% in the western regions[15,17]. Nonetheless, a study conducted by Baseri *et al.*[18]. did not detect any *C. burnetii* infections using molecular techniques. Conversely, research by Lyytikäinen *et al.*[19] and Javad and Mohammad[20] reported a significant prevalence of *C. burnetii* among women who had experienced abortions, particularly in rural populations.

In the current study, the highest percentage of abortions occurred during the first trimester (54%), while the lowest was reported in the third trimester (18.0%). Acute Q fever infection during the first trimester significantly increases the risk of both maternal and fetal complications, including progression to chronic Q fever. Asymptomatic infections during pregnancy can evolve into chronic conditions, thereby raising the risk of reinfection in subsequent pregnancies[21,22]. A study conducted in Denmark demonstrated a significantly higher seroprevalence rate of Q fever (47%) among pregnant women exposed to livestock compared to non-exposed women (4.8%)[23,24].

Coxiella burnetii infection was identified exclusively through real-time PCR in 4% of the total participants—women who had experienced abortions in Thi-Qar province. Several investigations have employed molecular methods to determine the prevalence of *C. burnetii* in abortion specimens from pregnant women. A study conducted in Turkey, which analyzed 51 placental samples, reported no evidence of *C. burnetii* infection[25]. Similarly, a study in France examining 246 placental samples also found no presence of the pathogen[26]. These findings contrast with the results of the present study. An investigation in Algeria reported a low positive incidence of *C. burnetii* in placental samples (0.55%)[7,27].

A study by Ateya (2017) in Iraq found that 17.02% of blood specimens from women who had experienced abortions tested positive for *C. burnetii* using PCR techniques[14]. In addition to molecular approaches, several studies have assessed the frequency of Q fever in pregnant women using serological tests. Two investigations from Turkey reported Q fever seroprevalence rates of 20.7% and 14%, respectively[25].

The detection of Q fever has also been explored in countries neighboring Iraq, such as Turkey and Iran. A serosurvey conducted by Kennerman *et al.*[28]. on 42 sheep flocks in Turkey found that 20% of the animals were seropositive for *C. burnetii*. Likewise, studies conducted in Iran by Khalili and Sakhaee[29] and Khalili *et al.*[30]. provided serological evidence confirming exposure to *C. burnetii* among both human and animal populations.

CONCLUSION

The real-time PCR technique is a critical and highly accurate method for detecting *Coxiella burnetii* in women who have experienced abortions—particularly in cases that have not been clinically diagnosed in hospitals within Thi-Qar province, Iraq. These findings suggest that Q fever may be associated with a significant number of abortion cases in the region.

Author contributions

All authors had seen and approved the submission of the manuscript with full responsibility, and this research had not been published or under consideration by any other journal.

Conflict of Interest:

The authors declare that they have no conflict of interest.

Financial disclosure

The authors deny receiving any financial support or grant from any organization

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