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Molecular Analysis of Pregnancy Failure Due to *Coxiella Burnetii* (Coxielosis) in Women from Kut City

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Abstract: The current research focused on investigating the occurrence of Coxiella burnetii infections by analyzing the frequency of positive cases in particular age groups and geographical areas. Encouraging findings were observed among individuals aged 21-26 years, highlighting a significant detection rate and emphasizing the need for further investigation into infection susceptibility and preventive measures in this age range. Similar trends were noticed in other age intervals, underlining the importance of raising awareness and implementing targeted prevention strategies across different age brackets. Furthermore, variations in infection rates between urban and rural areas suggest differing transmission risks, possibly attributed to environmental factors and occupational exposures. Various studies have identified risk factors such as contact with infected animals and specific animal reservoirs. Pregnant women are particularly vulnerable to Coxiella burnetii infections, emphasizing the significance of considering regional factors and implementing preventive measures. Overall, this research underscores the need for further investigation and the implementation of targeted prevention strategies to mitigate the risk of Coxiella burnetii infections and improve public health outcomes.

Key points: Q fever, Abortion, Polymerase chain reaction, Wasit province, Iraq.

Introduction

Coxiella burnetii is a small bacterium that lacks the ability to survive outside of host cells. It is classified as Gram-negative and is considered an obligate intracellular parasite, meaning it requires host cells to survive and replicate, which means it can only survive and grow inside the cells of other organisms. *C. burnetii* was first reported as a disease in 1935 by Australian and American investigators (1). Q fever is a disease that can be spread to humans from animals. It has been reported in all countries in the world except for New Zealand (2). *Coxiella burnetii* has been detected in various organisms, including humans, livestock, domesticated animals, wildlife, and arthropods (3). *Coxiella burnetii* bacteria primarily infect and reside in farm animals, particularly dairy cattle, sheep, and goats, which serve as the main hosts for this bacterium.*C. burnetii* infection commonly manifests with characteristic symptoms in these animals, and they are often naturally infected. Infected animals, such as cattle, sheep, and goats, can experience reproductive disorders, and in the case of sheep and goats, it may even result in abortion (2, 4).

In the early or acute stage of the disease, *Coxiella burnetii* bacteria can be detected and isolated from the blood, lungs, spleen, and liver of the infected individual.Though, in the chronic phase of the disease, the primary sites of infection shift to the uterus and mammary glands (2, 4), birth products, urine, semen, feces, vaginal fluids, placenta, and aborted fetuses of diseased animals.A significant source of *Coxiella burnetii* bacteria in the environment primarily occurs during the process of parturition (birth), (5). There is well-documented evidence that the placenta of sheep and

the milk produced by infected dairy cattle can contain *Coxiella burnetii* bacteria. This indicates that these animals can transmit the bacterium through these specific routes (2, 4).*Coxiella burnetii* infection in livestock can cause abortion and decreased reproductive efficiency. Infection is often asymptomatic (5, 6).

In humans, Q fever between 2007-2009 was the largest-ever outbreak reported in the Netherlands, involving over 3,500 human cases that led to renewed attention (7). Recent studies (8, 9) have provided confirmation that *C. burnetii* is a causative agent of febrile illness and community-acquired pneumonia, particularly in resource-limited settings. In a study conducted in northern Tanzania, where the etiology of fever among hospitalized patients was investigated, researchers discovered that Q fever, caused by *C. burnetii*, was more prevalent as a cause of severe febrile illness compared to malaria (10, 11). Due to the success of control programs in sub-Saharan Africa, which have resulted in significant reductions in malaria cases (11, 12), new public health priorities are now emerging. These priorities emphasize the need for improved diagnosis, treatment, and control of non-malaria febrile illnesses, including Q fever. As malaria incidence decreases, efforts are being redirected towards addressing other febrile illnesses to further enhance public health outcomes in the region (11). In addition, *C. burnetii* is one of important pathogen responsible for disease in human, indirectly, can causes socioeconomic through decrease livestock productivity (13). In a study carried out by Gharban and Yousif in Iraq in 2021, a significant milestone was achieved as *Coxiella burnetii* was isolated for the first time from lactating cows. This finding adds to the understanding of the presence and potential impact of *C. burnetii* in the bovine population in that particular region.The study confirmed that the organism was actively shed through milk, indicating that these animals play a significant role in the transmission of the disease to humans and other animal species. The findings suggest that lactating cows can serve as reservoir hosts for *Coxiella burnetii* (14).

Materials and methods Samples

This study was included totally 48 women who aged 21-37 years, were attended to Al-Zahraa Teaching Hospital during February to May 2022, due to pregnancy failure. Each one was subjected for draining 2.5 ml of venous blood samples under aseptic conditions into a labeled EDTA-plastic tubes that kept frozen at -20ºC until be tested.

Molecular examination

In the laboratory, the process of thawing whole blood samples involved placing the tubes in a water bath at 37 ºC and vigorously shaking them using a vortex machine to ensure proper mixing. Following the manufacturer's instructions (Intron, Korea), the DNA extraction protocol (A) was followed. To quantify the extracted DNA, a Nanodrop spectrophotometer (ThermoScientific, USA) was utilized following the guidelines provided by the manufacturer. The Nanodrop spectrophotometer is commonly used for measuring the concentration and purity of nucleic acid samples.A 2 µl volume of the chromosomal DNA was placed on the spectrophotometer's pedestal, and the absorbance of the sample at 260 nm was measured to determine the DNA concentration in ng/µl. The purity of the DNA was evaluated by determining the ratio of absorbance at 260 nm and 280 nm. This ratio is commonly used as an indicator of DNA quality, with a higher value indicating better purity and absence of contaminants.

To amplify the *C. burnetii* DNA targeting the *16S rRNA gene*, a specific set of primers (F: 5'- AGT ACG GCC GCA AGG TTA AA-3' and R: 5'-CTC CAA TCC GGA CTA CGA GC- 3') were used. For each primer, the PCR mastermix was prepared using the AccuPower PCR-

PreMix kit (Bioneer, South Korea), with a final volume of 20 µL. The PCR reaction was carried out in a Thermocycler (Bio-Rad, USA) under the following conditions: an initial denaturation step (5 min / 95ºC), followed by 30 cycles of denaturation (40 sec / 95ºC), annealing (40 sec / 56ºC), and extension (1 min / 72ºC), and a final extension step (7 min / 72ºC). To analyze the PCR products, 10 µL of each sample was loaded onto a 2% agarose gel stained with Ethidium Bromide (Biotech, Canada), and a DNA marker with a range of 100-2000 base pairs (Qiagen, Germany) was used as a

size reference. Electrophoresis was conducted at 100 volts and 80 amperes for 1 hour, and the amplified DNA products were visualized under a UV transilluminator (Clinx Science, China).

Results

In precent study examining positive cases of *Coxiella burnetii* within specific age intervals, encouraging findings were observed among individuals aged 21-26 years (Table 1). Out of a total of 20 patients within this age group, 4 were found to be positive for *Coxiella burnetii*. This indicates a significant detection rate, highlighting the need for further investigation into the factors influencing infection susceptibility and the effectiveness of preventive measures in this specific age range. Similar trends were also noticed in other age intervals, with 27-31 years showing 13 patients and 2 confirmed *Coxiella burnetii* infections, while the 32-37 age group had 15 patients and 1 positive case. These findings underscore the importance of raising awareness and implementing targeted prevention strategies to mitigate the risk of *Coxiella burnetii* infections across different age brackets.

Table 1: distribution of patient number regarding age and positives cases

Age	Number	Positive
21-26	20	4
27-31	13	2
32-37	15	1
Total	48	7

The incidence of positive cases of *Coxiella burnetii*d varied between different locations, with notable differences observed between a city and a village (Table 2). In the city, where 15 cases were reported, none of the individuals tested positive for *Coxiella burnetii*. This may suggest a lower prevalence or transmission rate of the bacterium within urban settings, possibly due to differences in environmental factors, population density, or occupational exposure. On the other hand, in the village, where 33 cases were identified, 7 individuals tested positive for *Coxiella burnetii*. This indicates a higher rate of infection in the rural environment, potentially associated with factors such as close proximity to livestock, agricultural activities, or other sources of exposure to the bacterium. These findings highlight the importance of considering local factors and specific geographical locations when assessing the risk and implementing preventive measures against *Coxiella burnetii* infections.

Table 2: distribution of patient number regarding area and positives cases

Area	Numbers	Positive
City	15	0
Village	33	7
Total	48	7

The representative image (Figure 1) shows an agarose gel electrophoresis of PCR products targeting the 16S rRNA gene, resulting in a specific band at 425 base pairs (bp). The gel includes a ladder marker (M) that ranges from 2000 to 100 bp, which serves as a reference for the size of the DNA fragments. Out of the total samples tested, 7 (14.58%) were found to be positive for *Coxiella burnetii*. The positive control, which validates the effectiveness of the PCR assay, is represented in Lane 5. The negative control, which should not yield any amplification product, is shown in Lane 14.

The positive samples, indicating the presence of *Coxiella burnetii*, are observed in Lanes 1, 2, 4, 7, 9, 11, and 12. On the other hand, the negative samples, indicating the absence of *Coxiella burnetii*, are seen in Lanes 3, 6, 8, 10, and 13.

These results suggest that among the women who experienced pregnancy failure and were tested in this study, approximately 14.58% showed molecular evidence of *Coxiella burnetii* infection. It is important to note that further analysis and interpretation of these results, along with clinical correlation, would be necessary to establish a definitive diagnosis and understand the implications of *Coxiella burnetii* in pregnancy failure cases.

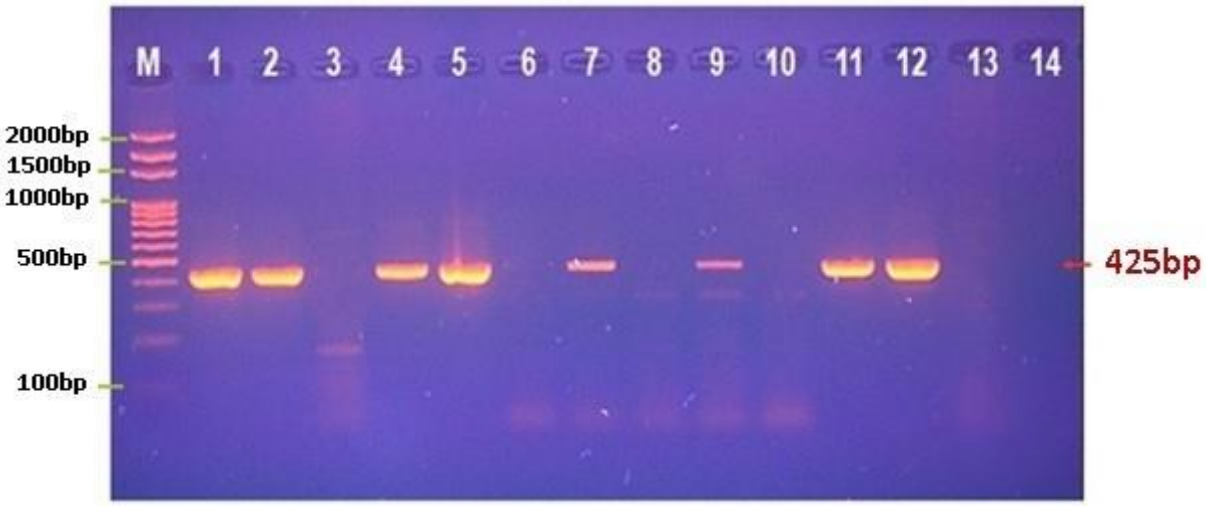

Figure 1: Representative image for agarose gel electrophoresis of PCR products targeting *16S rRNA* gene at 425 bp

Discussion

Coxiella burnetii is a bacterium known to cause Q fever in both humans and animals. The present study focused on investigating the prevalence of positive *Coxiella burnetii* cases within specific age intervals. Encouraging findings were observed among individuals aged 21-26 years, with 4 out of 20 patients in this age group testing positive for the bacterium.Similar trends were also noticed in other age intervals, with 27-31 years showing 13 patients and 2 confirmed *Coxiella burnetii* infections, while the 32-37 age group had 15 patients and 1 positive case. These findings highlight the need for further investigation into the factors influencing infection susceptibility and the effectiveness of preventive measures in these specific age ranges. However a study by Hamzić *et al* find that the highest prevalence was found in individuals aged 31-40 years (15). The high highest percentage differences in our study and Hamzić *et al* study may due the age of workers and extended age of working in other study compared to Iraq.

The incidence of positive cases of *Coxiella burnetii* varied between different locations, with notable differences observed between a city and a village. In the city, where 15 cases were reported, none of the individuals tested positive for *Coxiella burnetii*. On the other hand, in the village, where 33 cases were identified, 7 individuals tested positive for *Coxiella burnetii*. This may suggest a lower prevalence or transmission rate of the bacterium within urban settings, possibly due to differences in environmental factors, population density, or occupational exposure. Conversely, this indicates a higher rate of infection in the rural environment, potentially associated with factors such as close proximity to livestock, agricultural activities, or other sources of exposure to the bacterium (16). These findings highlight the importance of considering local factors and specific geographical locations when assessing the risk and implementing preventive measures against *Coxiella burnetii* infections.

Other studies have also examined risk factors associated with *Coxiella burnetii* infections. For example, a study in Iraq found that lactating cows can be a source of *C. burnetii* infection, with a prevalence rate of 18.46% detected in milk samples (14). In a study conducted in Iran, the seroprevalence of anti-*Coxiella burnetii* IgG antibodies was investigated among industrial slaughterhouse workers. The results of the study showed that 56% of the individuals included in the study tested positive for anti-*Coxiella burnetii* antibodies. This indicates that a significant portion of the study population has been exposed to the bacterium at some point, as the presence of these

antibodies is indicative of past exposure (17). The study demonstrated that individuals who had a history of accidental hand cuts and those who primarily handled sheep as their livestock had higher odds of Q fever infection. This suggests that these specific factors, such as hand cuts and close contact with sheep, contribute to an increased risk of contracting Q fever (17). A study conducted in Bosnia and Herzegovina revealed that Q fever is a looming public health concern in the region. The study identified specific anti-C. burnetii antibodies in 249 individuals, which accounted for approximately 35.2% of the population studied. These findings highlight the presence of Q fever in the area and emphasize the need for proactive measures to address this emerging public health issue (15). In a study conducted in Iraq, the prevalence of chronic infections with *C. burnetii* was estimated in humans and sheep. The study found that the overall prevalence of *C. burnetii* phase-1 was 7.38% among the samples that tested positive for antibodies. Specifically, the prevalence was 3.3% in humans and 11.41% in sheep. These results indicate a higher prevalence of *C. burnetii* phase-1 in sheep compared to humans, suggesting that sheep may serve as a significant reservoir for the bacterium in the studied population (18). Significant increases in sero-positive results were seen among rural areas, older individuals, and female sheep (18).

Coxiella burnetii is a type of bacteria that can cause a zoonotic infection known as Q fever in humans. Transmission of *Coxiella burnetii* to humans can occur through various routes, including inhalation of contaminated aerosols, ingestion of contaminated food or water, or direct contact with infected animals. While primarily a pathogen of animals, *Coxiella burnetii* can still pose a risk to human health under certain circumstances. It is crucial to be aware of these potential modes of transmission and take appropriate precautions to minimize the risk of infection.Pregnant women who become infected with *C. burnetii* are at risk of experiencing adverse pregnancy outcomes.

These may include complications such as abortion (miscarriage), intrauterine growth retardation (poor fetal growth), premature birth, oligoamnios (reduced amniotic fluid), and spontaneous abortion. It is important for pregnant women to take necessary precautions to prevent *C. burnetii* infection and seek appropriate medical care if they suspect exposure or develop symptoms (19, 20). The seroprevalence of *C. burnetii* infection among pregnant women can vary across different regions and populations. In a study conducted in southwestern and northern Iran, it was observed that the overall prevalence of *C. burnetii* among pregnant women was 29.3%. Notably, the prevalence was significantly higher in women who had a history of abnormal pregnancies. These findings underscore the significance of considering *C. burnetii* infection in pregnant women, especially those with a history of pregnancy complications. It highlights the need for appropriate monitoring and management to ensure optimal maternal and fetal health in such cases (19). Another study conducted in rural pregnant women in Khorramabad, western Iran, found that 48.4% of the serum samples were positive for C. burnetiid (21). In the Netherlands, a significant Q fever outbreak took place from 2005 to 2012. The outbreak initially began with the diagnosis of nearly one hundred patients who had lower respiratory tract infections caused by *C. burnetii*. The outbreak subsequently spread and became a major public health concern during that period (19). During the Q fever outbreak in the Netherlands, a seroprevalence study was carried out, revealing that the highest seroprevalence of *C. burnetii* was observed among pregnant and periparturient dairy goats in the south-eastern part of the country. This suggests that the infection was particularly prevalent in this specific population of goats during that period. Understanding the dynamics of *C. burnetii* transmission in animal populations is crucial for implementing effective control measures to prevent further outbreaks and mitigate the risk of transmission to humans.This indicates that these goats were particularly susceptible to infection and played a significant role in the transmission of *C. burnetii* during the outbreak (19). Bulk tank milk (BTM) testing conducted using real-time PCR and ELISA demonstrated a higher proportion of positive samples for *C. burnetii* in the southeastern part of the Netherlands compared to other regions of the country. This indicates a higher prevalence of the bacterium in that specific area.In a nested case-control study within the Danish National Birth Cohort, no evidence was found to suggest a higher prevalence of *C. burnetii* antibodies in serum samples from women who experienced miscarriages. However, it is important to note that the study did not include very early first-trimester abortions. Further research may be needed to explore the

potential association between *C. burnetii* infection and very early miscarriages in pregnant women (19).

Conclusion

Coxiella burnetii infections in different age intervals and locations highlight the need for further investigation into the factors influencing infection susceptibility and the effectiveness of preventive measures. While the study observed a significant detection rate among individuals aged 21-26 years, it is important to consider variations reported in other studies and regional factors. The differences in prevalence between urban and rural areas suggest varying levels of exposure and transmission risks. Risk factors such as contact with infected animals and specific animal reservoirs have been identified in other studies. Additionally, pregnant women are particularly susceptible to adverse outcomes. To mitigate the risk of *Coxiella burnetii* infections, targeted prevention strategies should be implemented, taking into account regional factors and vulnerable populations such as pregnant women. Additional research is required to advance our comprehension of this bacterium and enhance public health outcomes.

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