The incidence of Coxiella infection in Iraqi women with early pregnancy loss
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Abstract:
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This study looks at how serologic results of Q fever and early pregnancy loss (EPL) are linked. In Baghdad, including Madinat Al-Imamain Al-Kadhmain Teaching Hospital and Abu Ghraib Hospital, multicenter case-control research was conducted from September to December 2022. A total of 90 women were enrolled in this investigation. Sixty clinically suffered from early pregnancy loss by having a nonviable intrauterine pregnancy. The other 30 women were subjects of comparable age, the week of pregnancy, and the healthy subjects as controls. Using a commercial enzyme-linked immunosorbent assay (ELISA), serum samples were screened for antibodies against CB. Three (5%) cases tested positive for CB by ELISA. The study reveals no association between adverse gestation outcomes and positive Q fever serology. Age and abortion history were not significantly correlated with C. burnetii seropositivity, according to the findings of CB cases that tested positive results.

Keywords: Query fever; Coxiella burnetii; Early pregnancy loss; Enzyme-linked immunosorbent assay; Iraq

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Introduction
Coxiella burnetii (CB) is a Gram-negative, pleomorphic, strictly intracellular, 0.4–1.0m and 0.2–0.5m long and wide bacterium. It is classified as Bacteria, Proteobacteria, Gammaproteobacteria, Legionellales, Coxiellaceae, Coxiella, genus Coxiella, and species CB (1–3).

Humans can incubate CB for 14–28 days or longer depending on the inoculation dose, infection method, and antigenic phase. This pathogen's cell wall demands a structurally and antigenically unique LPS molecule (3). Based on structure, LPS contains Phase II and Phase I antigenic variants. Infected hosts' virulent CB Phase II features a shortened LPS molecule without an O-antigen. Phase-I fully virulent LPS. Phase II involves multiple embryonated egg or cell culture passages. Chromosomal deletions in a 38-kb CB genomic region produce LPS antigenic phase variation (4). Two morphological varieties of CB exist: the large cell variant (LCV) and the small cell variant (SCV), which may be seen under an electron microscope. SCV is little and dormant, while LCV is large and metabolically active. SCV resists stress of the environment and has a prolonged survival rate in harsh environments (4). CB infections, commonly known as coxiellosis in animals and Query fever in humans, are a major cause of abortions, decreased reproductive efficacy, and subclinical infections in ruminants (5).

Q fever is a global zoonosis caused by CB, a Rickettsiaceae intracellular bacterium. CB is phylogenetically separate from the rest of this family, as shown by molecular biology (6). In 1955, Q fever was documented in 51 countries on five continents; four decades later, the disease was reported in an additional eight nations. In addition, seroepidemiological surveys have revealed the presence of Q fever in some other nations (7). Q fever epidemiology varies widely. Most European countries neglect Q fever because of its rarity. Molecular epidemiology and evolution of this disease in ruminants need additional study. Genomic research will suggest outbreak causes. Pathogenesis of CB requires molecular studies. Pasteurizing dairy milk and improving awareness may prevent Q fever-zoonosis (8) (one of the treatments use for Q fever infection is Amoxicillin which contain 2- azetidinone compounds that acts as broad spectrum antibiotic against gm +ve and gm-ve) (9).

The nomenclature classifies pregnancy losses as elective abortion (10), pre-embryonic loss (11), early and late fetal death (12), recurrent pregnancy loss (13), ectopic pregnancy (14), early pregnancy loss (15), and early and late fetal death (12). By utilizing the postmenstrual age, the gestational age can be determined. EPL is spontaneous loss of pregnancy prior to 13 weeks of gestation (16). Before clinical confirmation, many pregnancies are lost, 15% of clinically proven pregnancies end in spontaneous abortion, and 30% of conceptions result in live births (17). The European Society for Human Reproduction Special Interest Group for Early Pregnancy defines early gestation loss differently to promote consistency (15). In Table 1, Reclassification of early pregnancy loss are summarized.
Table 1: Reclassification of early pregnancy loss (18).

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy of unknown location (PUL)</td>
<td>Scan negative for pregnancy with positive hCG</td>
</tr>
<tr>
<td>Late pregnancy loss</td>
<td>Loss of FCA during a gestational age more than 12 weeks</td>
</tr>
<tr>
<td>Early pregnancy loss</td>
<td>Scan demonstrating an empty sac or a sac containing a fetus but no FCA (pregnancy lasting less than 12 weeks)</td>
</tr>
<tr>
<td>Biochemical pregnancy loss</td>
<td>Pregnancy not found by ultrasound</td>
</tr>
<tr>
<td>Fetal loss</td>
<td>Previous CRL measurement with the subsequent absence of fetal cardiac activity (FCA)</td>
</tr>
<tr>
<td>Empty sac</td>
<td>Ultrasound showing sac without any structure or minimal structures</td>
</tr>
</tbody>
</table>

When describing a miscarriage diagnosis in the medical record, use exact and suitable terminology. Current use has been criticized for obscurity and misunderstanding (19–21). 50–60% of EPL instances are due to conceptus chromosomal insufficiency, while up to 90% of cases with embryonic mortality early in gestation have an aberrant chromosome complement (22). Less than 10% of these disorders involve genetic or structural abnormalities (23). Unsurprisingly, older mothers had a five-to-seven-fold higher miscarriage risk (24). The likelihood of abnormalities of numerical chromosomal occurring again is low, and 2% of early miscarriages result in a trisomy (25). However, a couple with repeated pregnancy losses and a normal conceptus karyotype is likely to miscarry (26–28). Other reasons for miscarriage infections (Trichomoniasis is one of infections that causes early pregnancy loss) (29), chemical agents, immunological factors, psychological factors, inherited conditions, trauma, and maternal illnesses. Worldwide, 23 million miscarriages occur annually, 44 every minute (30).

Serology is the main laboratory test for Q fever. Serological assays use inactivated whole cells from phase I and phase II CB strains. Nine Mile phase I (RSA 493) is a CB reference strain (31). The present study aimed to assess the Q fever level of Iraqi women with early pregnancy loss, and the relationship between Q fever serologic results and (EPL) in a population of women with symptomatic pregnancy.

Patients and Methods
In Baghdad, including Madinat Al-Imamain Al-Kadhmain Teaching Hospital and Abu Ghraib Hospital, multicenter case-control research were conducted from September to December 2022. A total of 90 women were enrolled in this investigation. Sixty of them were clinically suffering from early pregnancy loss by having a nonviable intrauterine gestation that happens within rather 12–7 weeks of pregnancy and has either an empty gestational sac or one with an embryo or fetus inside. Still, no fetal cardiac activity (FCA) or has vaginal bleeding. The other 30 women were subjects of comparable age, week pregnancy, and healthy subjects as controls.
Ethical Approval
The Mustansiriyh University College of Pharmacy's Ethical Committee gave its approval to this study. Before the agreement to participate in the study was recorded, all participants were made aware of its goals and expected advantages.

Inclusion Criteria
Reproductive-age women from [18 to 38] years old for both patient and control women.

Exclusion Criteria
Women with chronic disease, such as: (Hypertension, Diabetic, Heart disease, Hyperthyroidism disease, Kidney disease, Arthritis, Stroke, Asthmatic patient, Autoimmune disease, Patients with toxoplasmosis, Patients with CMV, patient with mycoplasma infection, women of age below 18 years old, and above 38 years old.

Specimens
Five mL of venous blood specimen was obtained from each participant, which was placed in a gel tube and then left for an appropriate time (nearly 15-30 min) for allowing them to clot at 25°C followed by centrifugation at 4400 (rpm) for fifteen min. The obtained serum was divided into two tubes, the first one also divided into two groups for rapid detection of CMV and toxoplasma to exclude the sample and if the sample is free from these two pathogens the second part of serum was frozen below (-20) °C until it was used in the analysis of mycoplasma IgG, Coxiella IgG The analysis manner of mycoplasma IgG, Coxiella IgG. were summarized Figure 1.

![Diagram](image-url)
Serological tests
It was opted for a one-step procedure to identify anti-CB antibodies. A commercial enzyme-linked immunoassay assay (ELISA) was utilized to analyze each sample. The ELISA reagent was purchased from (MYBIOSOURCE, USA) and used following the manufacturer's instructions. Consider that some studies publish their findings based exclusively on ELISA when evaluating the relationship between spontaneous abortion and Q fever titers (32).

Statistical analysis
The V24 of SPSS software was used for statistical data evaluation. This study utilized descriptive analysis, which will provide ‘summary’ statistics such as mean, median and standard deviation. Descriptive statistics summarize facts and information numerically and graphically. Numerical techniques include measures of the central tendency and measures of variability. Descriptive analysis was used to examine variables by the group ( t-test and Chi-square test). The ordinal logistic regression technique evaluated the association strength between one dependent variable (number of pregnancy weeks) and independent variables (age, the group, coxiella burnetti). Goodness-of-Fit statistics were utilized for determining whether the model adequately describes the data (33).

RESULTS
The research population demographic characteristics are shown in Table 2. The women in both groups (patient= PW) and (control= CW) fell in the age group of 20–38 years old. Maternal age (by t-test) for women who experienced patient women PW presented at a significantly higher age than women (control) CW (the mean CW age:28.33 (yrs) 95% CI (26.42 – 30.25); the mean PW age: 28.82 (yrs) 95% confidence interval of mean (95%CI) 27.09 – 30.54 (P value:0.687) Figure 2a. For gestational age (by t-test), (mean CW gestational age:8.70 (weeks) 95% CI (7.41 – 9.99); mean PW gestational age: 9.47 (weeks) 95% CI (8.83 – 10.10) (P value:0.823) Figure 2b. For No. of children (by t-test), (mean PW children: 3.65 95% CI (2.99 – 4.31); mean CW No. of children: 2.80 (weeks) 95% CI (1.99 – 3.61). Among the groups, statistically, (P value:0.103), Figure 2c.

Table 2 compares the median Coxilla serum levels in PW versus CW. Serum Coxilla levels in PW were (median .9500, 95% CI (.8932 – 1.0068) while the CW group Coxilla is constant when group = control and it has been omitted (P value:0.291) by Chi-square test. By indirect ELISA test after the addition of substrate to the wells any well get blue Color indicate positive presence of coxiella antigen. Both phase I and phase II antibody titers are often 1:128 or higher in Q fever samples. IgG class antibody titers occur early in the disease, peaking stage II by week 8 and lasting over a year. The number of positive and negative Coxilla analyses for the patient and control group were depicted in Figure 3.
Table 2: Descriptive analysis (examine variables by the group).

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>95% confidence Interval of Mean</th>
<th>Median</th>
<th>Std. Deviation</th>
<th>Min.</th>
<th>Max.</th>
<th>Range</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>patients</td>
<td>60</td>
<td>28.82</td>
<td>27.09</td>
<td>30.54</td>
<td>28.50</td>
<td>6.668</td>
<td>20</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>30</td>
<td>28.33</td>
<td>26.42</td>
<td>30.25</td>
<td>27.50</td>
<td>5.135</td>
<td>20</td>
<td>38</td>
</tr>
<tr>
<td>No. of children (G)</td>
<td>patients</td>
<td>60</td>
<td>3.65</td>
<td>2.99</td>
<td>4.31</td>
<td>3.00</td>
<td>2.570</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>30</td>
<td>2.8</td>
<td>1.99</td>
<td>3.61</td>
<td>3.00</td>
<td>2.156</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Week of Pregnancy</td>
<td>patients</td>
<td>60</td>
<td>9.47</td>
<td>8.83</td>
<td>10.10</td>
<td>10.00</td>
<td>2.446</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>30</td>
<td>8.70</td>
<td>7.41</td>
<td>9.99</td>
<td>10.00</td>
<td>3.456</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Coxiella burnetii</td>
<td>patients</td>
<td>60</td>
<td>0.9500</td>
<td>0.8932</td>
<td>1.00681</td>
<td>1.00</td>
<td>0.21978</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>1.00</td>
<td>a</td>
<td>1.00</td>
<td>a</td>
</tr>
</tbody>
</table>

*a Coxilla burnetii is constant when group = control.

It has been omitted.
Figure 2. Stem-and-Leaf Plot for patient and control women (a) age (pvalue:0.687), (b) the week of pregnancy (pvalue:0.823), and (c) No. of the children (pvalue:0.103).

![Stem-and-Leaf Plot](image)

Figure 3. The positive and negative coxiella burnetii analysis.

The case processing summary is illustrated in Table 3, by using the ordinal logistic regression test; the higher marginal percentage of the week of pregnancy in this study was for 12, which was 37%, and the lower was 1.1% for the week of pregnancy of 13. The Marginal Percentage of the patient group was 66.7%, while for the control group was 33.3%. The positive Marginal Percentage was 3.3%, and the negative was 96.7%.

Table 3: Case Processing Summary (Ordinal logistic regression test)

<table>
<thead>
<tr>
<th>week of pregnancy</th>
<th>N</th>
<th>Marginal Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>4</td>
<td>4.4%</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>13.3%</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>7.8%</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>15.6%</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>6.7%</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>8.9%</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>4.4%</td>
</tr>
<tr>
<td>12</td>
<td>34</td>
<td>37.8%</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>1.1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>group</th>
<th>N</th>
<th>Marginal Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>patient</td>
<td>60</td>
<td>66.7%</td>
</tr>
<tr>
<td>control</td>
<td>30</td>
<td>33.3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coxillab</th>
<th>N</th>
<th>Marginal Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive</td>
<td>3</td>
<td>5.0%</td>
</tr>
</tbody>
</table>
Using goodness-of-fit statistics, one can determine whether or not a model adequately describes a data set Table 5. The significance value is displayed in the table (p > 0.05), indicating that the model satisfactorily fits the data. The term "goodness-of-fit test" is typically used to determine how closely the observed data conform to the presumed (fitting) model (34). In this instance, an insignificant finding would indicate that there are no significant deviations between the observed data and the model fitted to those data (measured).

Table 5. Goodness-of-Fit (for Ordinal logistic Regression Test)

<table>
<thead>
<tr>
<th></th>
<th>Chi-Square</th>
<th>df</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson</td>
<td>246.594</td>
<td>269</td>
<td>833</td>
</tr>
<tr>
<td>Deviance</td>
<td>170.910</td>
<td>269</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Link function: Logit.

The estimate, seen in Table 6, represents the probability of an instance falling into an associated category greater than the current category. The sign is understood to represent a linear regression. With the presence of a plus sign, the probability of an instance being assigned to a higherly category in the variable of interest increases. The presence of a negative sign is linked to an increased risk of a case being assigned to a lower category in the dependent variable. For age, a positive indicator with age (0.044) would indicate that there is a higher relationship with pregnancy from 6-12 weeks and a lower relationship with pregnancy 4-5 weeks as the individual's age increases.
Link function: Logit. Wop= week of pregnancy. Ch.= coxiella b.
1. OR = EXP(Estimate) will be used in next table.
   a. This value is set to 0 as it is unnecessary.

For the patient group, for age, a positive sign with the patient group (0.339) would be that patient there is a higher (relationship) with pregnancy from (6-12) weeks and lower for pregnancy 4-5 weeks. For coxilla b. (0.485) is the same for the patient. Table (7), The variables from ordinary logistic regression analysis. The findings revealed that age (OR = 0.0.1196, 95% CI: -0.17–0.105), the group (patient) (OR = 0.9215, 95% CI: -0.451–1.129), coxilla b. (patients) (OR = 1.318, 95% CI: -1.664–2.634) were identified as potential risk factors during pregnancy periods from (4-12) weeks. The value of (OR) was calculated as illustrated in Table 5 above.

Table (7) Odds ratio. (for the ordinal logistic regression test)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>OR</th>
<th>Std. Error</th>
<th>95% C. I.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td>.044</td>
<td>0.1196</td>
<td>.031</td>
<td>-.017–.105</td>
<td>.155</td>
</tr>
<tr>
<td>[group=0]</td>
<td>.339</td>
<td>0.9215</td>
<td>.403</td>
<td>-.451–1.129</td>
<td>.401</td>
</tr>
<tr>
<td>[group=1]</td>
<td>0’</td>
<td></td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>[Cb=.00]</td>
<td>.485</td>
<td>1.318</td>
<td>1.096</td>
<td>-1.664–2.634</td>
<td>.658</td>
</tr>
<tr>
<td>[Cb=1.00]</td>
<td>0’</td>
<td></td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

For the patient group, for age, a positive sign with the patient group (0.339) would be that patient there is a higher (relationship) with pregnancy from (6-12) weeks and lower for pregnancy 4-5 weeks. For coxilla b. (0.485) is the same for the patient. Table (7), The variables from ordinary logistic regression analysis. The findings revealed that age (OR = 0.0.1196, 95% CI: -0.17–0.105), the group (patient) (OR = 0.9215, 95% CI: -0.451–1.129), coxilla b. (patients) (OR = 1.318, 95% CI: -1.664–2.634) were identified as potential risk factors during pregnancy periods from (4-12) weeks. The value of (OR) was calculated as illustrated in Table 5 above.

Table 7 shows the probability of falling into a higher or lower dependent variable category for each value variation in the independent variable. These odds are represented as a ratio between the two variables. When odds ratio is greater than one, it indicates a greater likelihood of being placed in a higher category for each additional unit of the predictor. OR<1 implies that the odds of belonging to a higher category fall as the predictor increases by one unit. This is indicated by the odds decreasing as predictor increases.

The ROC curve with area under the curve (AUC) of these parameters were shown in Fig. 4. Its shown that AUC near 0.5 and 95% CI (0.44 – 0.650).
Discussion

The mounting evidence linking Q fever to bad gestation outcomes warrants screening in high-endemicity settings and treating infected mothers since Q fever is asymptomatic mainly (35). Some diseases enhance the likelihood of spontaneous abortions, although it's hard to prove (36). In this study, only three positive (Q-fever infection) cases could be classified as a probable chronic Q fever figure (2) ages 20-31 yrs old. This investigation found a week of pregnancy 9 -12 (week) from Abu Ghraib hospital. The mother's age is a significant factor affecting the probability of miscarriage (37). However, it is not reasonable to conclude that there is a correlation between age and the prevalence of C. burnetii infection (32). From the results, patient women (PW) presented at a significantly higher age than women (control) CW. Among the groups, there was a statistically considerable distinction in maternal age. It was found that there was a significant gestational age difference. Also, it was found that a significant number of children's differences existe.

This study investigated Coxiella burnetii(CB) antibodies in serum samples using the ELISA assay instead of conventional serological techniques because the ELISA has greater sensitivity, is easier to execute in laboratories, is less expensive, and is quicker. Additionally, the ELISA test has a larger throughput, meaning more samples may be tested simultaneously (33) in addition to Wegdam-Blans et al. recommendation, they did not recommend phase I antibody detection for Q fever serological diagnosis (38).

Positive and negative results may not rule out current or past infections. The indirect diagnostic technique ELISA recognizes antibodies to detect CB exposure. Regional and national seroprevalence differences may be related to certain variables, including environmental circumstances, the time of research, and administration procedures, all of which may impact the transmission of C. burnetii (33). The ELISA test distinguished between the IgG and IgM classes of antibodies to phase II antigen. The first blood antibody is IgM phase II, followed by IgG phase II. IgM and IgG phase II antibodies might last a year or more (32).
As found by Jalel A. et al. investigation, that they reported Q. fever is an occupational disease, a zoonotic disease affecting people in the province of Thi-qar (Nasiriyah City-Iraq (39). Three cases in abugarib (this study) in the rural area and the exposure to the livestock leads to infection with Q. fever. Also, it could not link C. burnetii seropositivity to a poor pregnancy outcome with the same results by Stine Y. N. et al.(40), who studied implications and risks in pregnancies with rising or positive titers , suggesting that Denmark's Q fever risk –associated adverse gestation outcomes is low (19). Julien J. et al. conducted a serosurvey to determine pregnant women's Coxiella burnetii infection, for 203 pregnant women. They found that Q fever serum test were seropositive after seven months and seropositivity did not predict poor perinatal outcomes(41). Obaidat et al. evaluated Coxiella burnetii seroprevalence and risk factors. They found that (the governorate of residency consuming of raw milk, sheep, goats, and dog ownership) strongly correlated with. This leads to what we found that rural area is C. burnetii seropositivity source (42).

The true positive and negative rates are the main indicators of diagnostic accuracy. Specificity (the real negative rate) is the likelihood that the test accurately labels a healthy patient as negative. Sensitivity is the probability that the test accurately labels a diseased patient as positive (43). An AUC of 1 suggests a reliable test, while ≤ 0.5 cannot discriminate positive samples from negative controls (44) comparing with results found that AUC closed to 0.5 which cannot discriminate positive samples from negative controls.

**Conclusion**

According to the present study, pregnant Iraqi women with a history of animal contact were found to have Q fever. Based on the findings, pregnant women do not have a significant cause for concern regarding spontaneous abortions induced by coxiella burnetii. However, Q fever remains a risk factor for pregnant women. Concerning the direction of future investigation on spontaneous abortion and coxiella burnetii, it would be pertinent to examine adverse gestation outcomes in a population of pregnant women who are highly exposed, such as female laborers and veterinarians with contact with livestock. Coxiella burnetii should be considered for febrile disorders by Iraqi farmers and veterinarians. In conclusion, there was no relationship between spontaneous abortion and raised antibody titers against coxiella burnetii during the first trimester.

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